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L3 ANSWER 1 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
2003:696406 Document No. 139:225508 Use of tumor up-regulated CARD-contg. antagonist of caspase 9 (TUCAN) cDNA for monitoring cancer prognosis and therapy. Reed, John C. (USA). U.S. Pat. Appl. Publ. US 2003165887 A1 20030904, 65 pp., Cont.-in-part of U.S. Ser. No. 388,221. (English). CODEN: USXXCO. APPLICATION: US 2002-141618 20020507. PRIORITY: US 1999-388221 19990901; US 2001-PV289233 20010507; US 2002-PV356934 20020212.

AB The invention provides methods for detg. a prognosis for survival for a cancer patient. One method involves measuring a level of a TUCAN in a neoplastic cell-contg. sample from the cancer patient, and comparing the level of TUCAN in the sample to a ref. level of TUCAN, wherein a low level of TUCAN in the sample correlates with increased survival of the patient. Another method involves measuring a level of TUCAN in a neoplastic cell-contg. sample from the cancer patient, and classifying the patient as belonging to either a first or second group of patients, wherein the first group of patients having low levels of TUCAN is classified as having an increased likelihood of survival compared to the second group of patients having high levels of TUCAN.

L3 ANSWER 2 OF 38 MEDLINE on STN
2003089462 Document Number: 22489093. PubMed ID: 12601057. Caspase activation in an experimental model of retinal detachment. Zacks David N; Hanninen Virve; Pantcheva Mina; Ezra Eric; Grosskreutz Cynthia; Miller Joan W. (Retina Service, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, Massachusetts, USA.. davzacks@umich.edu) . INVESTIGATIVE OPHTHALMOLOGY AND VISUAL SCIENCE, (2003 Mar) 44 (3) 1262-7. Journal code: 7703701. ISSN: 0146-0404. Pub. country: United States. Language: English.

AB PURPOSE: To test for apoptotic photoreceptor cell death and caspase activation as a function of time after induction of an experimental retinal detachment. METHODS: Retinal detachments were created in Brown Norway rats by injecting 10% hyaluronic acid into the subretinal space using a transvitreous approach. Light microscopy and terminal dUTP-biotin nick end-labeling (TUNEL) was performed at 1, 3, 5, and 7 days after detachment to assess for the morphologic features associated with apoptosis. Western blot analysis of retinal protein extracts was performed using **antibodies** against caspase-3, -7, and -9 and poly-ADP ribose-polymerase (PARP) at 1, 3, and 5 days after detachment. RESULTS: Light microscopic analysis of detached retinas showed the presence of pyknotic nuclei in the outer nuclear layer and disruption of the normal organization of the photoreceptor outer segments.

TUNEL-staining was positive in the outer nuclear layer only in the detached portions of the retina. Western blot analysis confirmed the time-dependent activation of caspase-3, -7, and -9 and PARP in the detached retinas. No morphologic stigmata of apoptosis or caspase activation was detected in attached retinas. CONCLUSIONS: The apoptotic photoreceptor cell death in experimental retinal detachments is associated with caspase activation.

L3 ANSWER 3 OF 38 MEDLINE on STN

2003102631 Document Number: 22502557. PubMed ID: 12616497. Perforin-dependent activation-induced cell death acts through caspase 3 but not through caspases 8 or 9. Chen Liane; Woo Minna; Hakem Razqallah; Miller Richard G. (Department of Medical Biophysics, University of Toronto and Ontario Cancer Institute, 610 University Avenue, Toronto, Ontario, Canada M5G 2M9.) EUROPEAN JOURNAL OF IMMUNOLOGY, (2003 Mar) 33 (3) 769-78. Journal code: 1273201. ISSN: 0014-2980. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB Activation-induced cell death (AICD) is a phenomenon in which activated T cells undergo apoptosis upon restimulation. We are studying a form of AICD that can occur before cells become competent to die by Fas (hence "early" AICD) and which depends on the presence of perforin. Previous studies indicate that it does not occur through granule exocytosis but via some endogenous pathway. We here investigate a possible role for caspases. Caspase 3(-/-) cells were protected, suggesting a role for caspase 3 in early AICD. After recrosslinking, caspase 3 activity could be detected in cell lysates between 3 and 12 h, and CD8(+) T cells became annexin V-positive between 15 and 18 h. Blocking anti-Fas ligand **antibody** failed to inhibit death, and no processing of either caspase 8 or caspase 9 was detected in recrosslinked cells. Furthermore, T cells lacking functional caspase 9 continued to die in early AICD. Thus, perforin-dependent early AICD appears to require activation of caspase 3, but not caspases 8 or 9. As perforin has no intrinsic catalytic abilities, we propose that it releases some endogenous activity that can activate caspase 3.

L3 ANSWER 4 OF 38 MEDLINE on STN

2003126407 Document Number: 22527533. PubMed ID: 12639677. Role of mitochondrial cytochrome c in cocaine-induced apoptosis in rat testes. Li Haikun; Xu Liping; Dunbar Joseph C; Dhabuwala C B. (Department of Urology, Wayne State University School of Medicine, Detroit, Michigan 48201, USA.) UROLOGY, (2003 Mar) 61 (3) 646-50. Journal code: 0366151. ISSN: 1527-9995. Pub. country: United States. Language: English.

AB OBJECTIVES: We have previously demonstrated that cocaine exposure leads to apoptosis in rat testes. To understand further the mechanism of cocaine-induced testicular damage, we studied the effect of cocaine on cytochrome c release from the mitochondria. We also determined the caspase 3, caspase 8, and caspase 9 activities in rat testes after chronic cocaine exposure. METHODS: Thirty-day-old male Sprague-Dawley rats received cocaine hydrochloride or equal volumes of normal saline subcutaneously daily for 90 days. The testes were removed at 15, 30, and 90 days of cocaine or saline administration. Mitochondria and cytosolic fractions from testes were isolated. Western blotting was performed in both fractions using anti-cytochrome c **antibody**. Caspase 3, caspase 8, and caspase 9 activities were determined by fluorometric assay. RESULTS: The expression of cytochrome c protein in the cytosolic fraction was increased on day 15 and persisted for up to 90 days after cocaine injection compared with controls. However, the expression of cytochrome c in testes was decreased in the mitochondria fraction on days 15, 30, and 90 after cocaine injections compared with the corresponding controls. The caspase activity study showed caspase 3 and caspase 9 activities increased in cocaine-treated testes at each point of the study compared with the corresponding controls. However, the caspase 8 activity in cocaine-treated testes did not change significantly at each point of the study compared with the corresponding controls. CONCLUSIONS: Our results suggest that the release of cytochrome c from mitochondria and its

subsequent activation of caspase 9 and caspase 3 in testes play a key role in cocaine-induced germ cell apoptosis. Our findings also indicate that cocaine-induced testicular germ cell apoptosis in rats is at least initiated through a mitochondria-associated pathway.

L3 ANSWER 5 OF 38 MEDLINE on STN

2003383954 Document Number: 22801418. PubMed ID: 12920191. Critical role for Akt1 in the modulation of apoptotic phosphatidylserine exposure and microglial activation. Kang Jing-Qiong; Chong Zhao Zhong; Maiese Kenneth. (Department of Neurology, 8C-1 UHC, Wayne State University School of Medicine, 4201 St. Antoine, Detroit, MI 48201, USA.) MOLECULAR PHARMACOLOGY, (2003 Sep) 64 (3) 557-69. Journal code: 0035623. ISSN: 0026-895X. Pub. country: United States. Language: English.

AB Biological targets for neurodegenerative disease that focus on the intrinsic maintenance of cellular integrity and the extrinsic prevention of phagocytic cellular disposal offer the greatest promise for therapeutic intervention. Protein kinase B (Akt1), a serine-threonine kinase closely involved in cell growth and survival, offers a strong potential to address both intrinsic and extrinsic mechanisms of neuronal injury. We demonstrate that overexpression of a constitutively active form of Akt1 (myristoylated Akt1) in differentiated SH-SY5Y neuronal cells provides intrinsic cellular protection against apoptotic genomic DNA destruction and membrane phosphatidylserine (PS) exposure. Transfection of SH-SY5Y cells with a plasmid encoding a kinase-deficient dominant-negative Akt1 eliminates cytoprotection, suggesting that activation of Akt1 is necessary and sufficient to prevent apoptotic destruction. Apoptotic neuronal membrane PS exposure provides a unique pathway for Akt1 to offer extrinsic cellular protection and block microglial activation, because independent cotreatment with an anti-PS receptor neutralizing **antibody** could also prevent microglial proliferation. Akt1 maintains nuclear DNA integrity and membrane PS exposure through the specific inhibition of caspase 3-, 8-, and 9-like activities that were linked to mitochondrial membrane potential and cytochrome c release. Our work elucidates a novel capacity for Akt1 to maintain cellular integrity through a series of cysteine protease pathways and to uniquely regulate microglial activation through the modulation of membrane PS residue externalization.

L3 ANSWER 6 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN

2002:833504 Document No. 137:358061 Conserved sequence of XIAP-binding motif in human caspase-9 and Smac/DIABLO and therapeutic uses for screening modulators of apoptosis. Alnemri, Emad S. (Thomas Jefferson University, USA). U.S. Pat. Appl. Publ. US 2002160975 A1 20021031, 52 pp., Cont.-in-part of U.S. Ser. No. 939,293. (English). CODEN: USXXCO. APPLICATION: US 2002-68569 20020206. PRIORITY: US 2001-PV267966 20010208; US 2001-939293 20010824.

AB The invention provides conserved sequence of XIAP-binding motif in human caspase-9 and Smac/DIABLO. The invention also provides caspase-9-related peptides and polypeptides capable of binding to an Inhibitor of Apoptosis Protein (IAP), as well as caspase-9 mutant that fail to undergo normal processing and fail to bind to an IAP. Nucleic acid mols., including expression vectors, encoding such peptides and polypeptides are also provided. Such peptides and polypeptides, are useful for inducing apoptosis and identifying inhibitors and enhancer of apoptosis.

L3 ANSWER 7 OF 38 MEDLINE on STN

2002417757 Document Number: 22161904. PubMed ID: 12171907. Combretastatin-A4 prodrug induces mitotic catastrophe in chronic lymphocytic leukemia cell line independent of caspase activation and poly(ADP-ribose) polymerase cleavage. Nabha Sanaa M; Mohammad Ramzi M; Dandashi Mahmoud H; Coupaye-Gerard Brigitte; Aboukameel Amro; Pettit George R; Al-Katib Ayad M. (Division of Hematology and Oncology, Department of Internal Medicine, Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, Michigan 48201, USA.) CLINICAL CANCER RESEARCH, (2002 Aug) 8 (8) 2735-41. Journal code: 9502500. ISSN: 1078-0432. Pub. country: United States. Language: English.

AB We have previously reported that combretastatin-A4 prodrug (CA4P), anantitubulin/antiangiogenic agent isolated from the South African willow tree Combretum caffrum, induced cell death primarily through mitotic catastrophe in a panel of human B-lymphoid tumors. In this study, we investigated the molecular aspects of the mitotic catastrophe and whether or not it shares the same pathways of apoptosis. For this we studied the effect of CA4P on selected markers of apoptosis [caspases 9 and 3, poly(ADP-ribose) polymerase (PARP), bcl-2, and bax] and G2-M protein regulators (p53, MDM2, 14-3-3sigma, GADD45, cdc2, cdc25, chk1, wee1, p21, and cyclin B1). The chronic lymphocytic leukemia cell line WSU-CLL was used for this purpose. Western blot analysis showed that 24 h of CA4P (5 nM) exposure induces caspase 9 activation and PARP cleavage. However, the addition of Z-Val-Ala-Asp-fluoromethylketone (a general caspase inhibitor) or Z-Leu-Glu(OMe)-His-Asp(OMe)-CH2F (a caspase 9 inhibitor) before CA4P treatment did not block cell death. No change in bcl-2 or bax protein expression was observed. Exposure of WSU-CLL cells to 4 and 5 nM CA4P was associated with overproduction of total p53 and no dramatic change in MDM2, 14-3-3sigma, GADD45, the cyclin-dependent kinase cdc2, its inhibitory phosphorylation, the cdc2-inhibitory kinase (wee1), chk1, or cdc25 hyperphosphorylation. The overaccumulation of p21 and cyclin B1 protein was obvious at 24 h. Furthermore, CA4P treatment showed an increase in the expression of a marker of mitosis (mitotic protein monoclonal-2 **antibody**) and an overaccumulation of the cyclin B in the nucleus. Our findings suggest that CA4P induces mitotic catastrophe and arrest of WSU-CLL cells mostly in the M phase independent of p53 and independent of chk1 and cdc2 phosphorylation pathways. Apoptosis is a secondary mechanism of death in a small proportion of cells through activation of caspase 9 and PARP cleavage. The two mechanisms of cell death, i.e., mitotic catastrophe and apoptosis, are independent of each other in our model.

L3 ANSWER 8 OF 38 MEDLINE on STN

2003014489 Document Number: 22395753. PubMed ID: 12507932. Boswellic acids trigger apoptosis via a pathway dependent on caspase-8 activation but independent on Fas/Fas ligand interaction in colon cancer HT-29 cells. Liu Jian-Jun; Nilsson Ake; Oredsson Stina; Badmaev Vladimir; Zhao Wan-Zhou; Duan Rui-Dong. (Cell Biology B, Biomedical Center, B11, Lund University, Sweden.) CARCINOGENESIS, (2002 Dec) 23 (12) 2087-93. Journal code: 8008055. ISSN: 0143-3334. Pub. country: England: United Kingdom. Language: English.

AB Boswellic acids are the effective components of gum resin of Boswellia serrata, which has anti-inflammatory properties. Recent studies on brain tumors and leukemic cells indicate that boswellic acids may have antiproliferative and apoptotic effects with the mechanisms being not studied in detail. We studied their antiproliferative and apoptotic effects on colon cancer cells and the pathway leading to apoptosis. HT-29 cells were treated with beta-boswellic acid (BA), keto-beta-boswellic acid (K-BA) and acetyl-keto-beta-boswellic acid (AK-BA), respectively. Apoptosis was determined by flow cytometry, by cytoplasmic DNA-histone complex and the activity of caspase-3. The cleavage of poly-(ADP-ribose)-polymerase (PARP) and expression of Fas were examined by western blot. Specific caspase inhibitors, polyclonal Fas **antibody**, and antagonistic Fas **antibody** ZB4 were employed to elucidate apoptotic pathways. DNA synthesis and cell viability were examined. Both K-BA and AK-BA increased cytoplasmic DNA-histone complex dose-dependently and increased pre-G(1) peak in flow cytometer analysis, with the effects of AK-BA being stronger than K-BA. BA only increased the formation of DNA-histone complex at a high concentration. K-BA and AK-BA increased caspase-8, caspase-9 and caspase-3 activities accompanied by cleavage of PARP. The effects of AK-BA on formation of cytoplasmic DNA histone and on caspase-3 activation were 3.7- and 3.4-fold, respectively, more effective than those induced by camptothecin. The apoptosis induced by AK-BA was inhibited completely by caspase-3 or caspase-8 inhibitor and partially by caspase-9 inhibitor. ZB4 blocked exogenous Fas ligand-induced apoptosis, but had no effect on

AK-BA-induced apoptosis. AK-BA had no significant effect on expression of Fas. Apart from apoptotic effect, these acids also inhibited [(3)H]thymidine incorporation and cell viability to different extent. In conclusion, boswellic acids, particularly AK-BA and K-BA have antiproliferative and apoptotic effects in human HT-29 cells. The apoptotic effect is mediated via a pathway dependent on caspase-8 activation but independent of Fas/FasL interaction.

L3 ANSWER 9 OF 38 MEDLINE on STN

2002079655 Document Number: 21648694. PubMed ID: 11698395. Apicidin, a histone deacetylase inhibitor, induces apoptosis and Fas/Fas ligand expression in human acute promyelocytic leukemia cells. Kwon So Hee; Ahn Seong Hoon; Kim Yong Kee; Bae Gyu-Un; Yoon Jong Woo; Hong Sungyoul; Lee Hoi Young; Lee Yin-Won; Lee Hyang-Woo; Han Jeung-Whan. (Department of Biochemistry and Molecular Biology, College of Pharmacy and Department of Genetic Engineering, College of Life Science and Natural Resources, Sungkyunkwan University, Suwon 440-746, Korea.) JOURNAL OF BIOLOGICAL CHEMISTRY, (2002 Jan 18) 277 (3) 2073-80. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB We previously reported that apicidin arrested human cancer cell growth through selective induction of p21(WAF1/Cip1). In this study, the apoptotic potential of apicidin and its mechanism in HL60 cells was investigated. Treatment of HL60 cells with apicidin caused a decrease in viable cell number in a dose-dependent manner and an increase in DNA fragmentation, nuclear morphological change, and apoptotic body formation, concomitant with progressive accumulation of hyperacetylated histone H4. In addition, apicidin converted the procaspase-3 form to catalytically active effector protease, resulting in subsequent cleavages of poly(ADP-ribose) polymerase and p21(WAF1/Cip1). Incubation of HL60 cells with z-DEVD-fmk, a caspase-3 inhibitor, almost completely abrogated apicidin-induced activation of caspase-3, DNA fragmentation, and cleavages of poly(ADP-ribose) polymerase and p21(WAF1/Cip1). Moreover, these effects were preceded by an increase in translocation of Bax into the mitochondria, resulting in the release of cytochrome c and cleavage of procaspase-9. The addition of cycloheximide greatly inhibited activation of caspase-3 by apicidin by interfering with cleavage of procaspase-3 and DNA fragmentation, suggesting that apicidin-induced apoptosis was dependent on de novo protein synthesis. Consistent with these results, apicidin transiently increased the expressions of both Fas and Fas ligand. Preincubation with NOK-1 monoclonal **antibody**, which prevents the Fas-Fas ligand interaction and is inhibitory to Fas signaling, interfered with apicidin-induced translocation of Bax, cytochrome c release, cleavage of procaspase-3, and DNA fragmentation. Taken together, the results suggest that apicidin might induce apoptosis through selective induction of Fas/Fas ligand, resulting in the release of cytochrome c from the mitochondria to the cytosol and subsequent activation of caspase-9 and caspase-3.

L3 ANSWER 10 OF 38 MEDLINE on STN

2003010612 Document Number: 22404679. PubMed ID: 12516968. Fas-mediated signaling enhances sensitivity of human soft tissue sarcoma cells to anticancer drugs by activation of p38 kinase. Li WeiWei; Bertino Joseph R. (Program of Molecular Pharmacology and Chemistry, Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center, New York, New York 10021, USA.) Mol Cancer Ther, (2002 Dec) 1 (14) 1343-8. Journal code: 101132535. ISSN: 1535-7163. Pub. country: United States. Language: English.

AB Sensitivity of human soft tissue sarcoma (STS) cells to methotrexate, doxorubicin, and paclitaxel was examined after cells were pretreated with CH-11, an agonistic anti-Fas **antibody**. A subtoxic dose (6 ng/ml) of CH-11 sensitized STS cells but not normal fibroblast cells to these anticancer drugs. CH-11 increased cytochrome c release and consequent activation of caspase-9, independent of caspase-8 and increased p38 activation. Addition of SB203580, a specific inhibitor of p38, resulted in a decrease in activation of this kinase and abrogation of

enhanced chemosensitivity (doxorubicin and paclitaxel) by CH-11. These results demonstrate that stimulation of the Fas pathway by a subtoxic dose of a Fas agonist can selectively enhance sensitivity of STS cells to certain chemotherapeutic agents through activation of p38.

L3 ANSWER 11 OF 38 MEDLINE on STN

2002719934 Document Number: 22369815. PubMed ID: 12481428. X-linked inhibitor of apoptosis (XIAP) blocks Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis of prostate cancer cells in the presence of mitochondrial activation: sensitization by overexpression of second mitochondria-derived activator of caspase/direct IAP-binding protein with low pl (Smac/DIABLO). Ng Chuen-Pei; Bonavida Benjamin. (Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles School of Medicine, Jonsson Comprehensive Cancer Center, University of California, Los Angeles, California 90095, USA.) Mol Cancer Ther, (2002 Oct) 1 (12) 1051-8. Journal code: 101132535. ISSN: 1535-7163. Pub. country: United States. Language: English.

AB The resistance to Apo2 ligand (Apo2L)/tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis could be overcome by treatment with subtoxic concentrations of actinomycin D (Act D) in prostate tumor cells. Furthermore, the sensitization to Apo2L/TRAIL-mediated apoptosis by Act D positively correlated with selective down-regulation of X-linked inhibitor of apoptosis (XIAP). In this study, we examined whether second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low pl (Smac/DIABLO), a known inhibitor of apoptosis (IAP)-neutralizing protein, sensitizes resistant prostate tumor cells to Apo2L/TRAIL-mediated apoptosis. The prostate tumor cell line CL-1 was treated with Apo2L/TRAIL, Act D, or a combination of the two. The apoptosis-mediated signaling pathway was examined by Western blotting and flow cytometry. Furthermore, CL-1 cells transfected with the anti-IAP inhibitor Smac/DIABLO were examined for sensitivity to Apo2L/TRAIL. Whereas Apo2L/TRAIL induced the release of cytochrome c and endogenous Smac/DIABLO in the CL-1 tumor cells, the cytosolic levels of both molecules were not sufficient to induce apoptosis. Transient transfectants with a Smac/DIABLO cDNA encoding a neutralizing inhibitor of IAPs were sensitized to Apo2L/TRAIL-mediated apoptosis. The sensitization to Apo2L/TRAIL by Smac/DIABLO overexpression was a result of synergistic activation of caspases-3, -9, and -8. Treatment of the Smac/DIABLO transient transfectant with Apo2L/TRAIL enhanced the release of Smac/DIABLO from mitochondria and led to reduction of IAP family proteins (XIAP, c-IAP1, and c-IAP2). These results show that Smac/DIABLO can sensitize CL-1 tumor cells to Apo2L/TRAIL-mediated apoptosis. Thus, up-regulation of Smac/DIABLO and sensitization to Apo2L/TRAIL-mediated apoptosis are of potential clinical application in the immunotherapy of drug-/Apo2L/TRAIL-resistant tumors.

L3 ANSWER 12 OF 38 MEDLINE on STN

2002080583 Document Number: 21665829. PubMed ID: 11807010. The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. Byrd John C; Kitada Shinichi; Flinn Ian W; Aron Jennifer L; Pearson Michael; Lucas David; Reed John C. (Division of Hematology-Oncology, The Ohio State University, B302 Starling Loving Hall, 320 W 10th Ave, Columbus, OH 43210, USA.. byrd-3@medctr.ohsu.edu) . BLOOD, (2002 Feb 1) 99 (3) 1038-43. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Rituximab is a chimeric monoclonal **antibody** directed at CD20 with significant activity in non-Hodgkin lymphoma (NHL) and chronic lymphocytic leukemia (CLL). A variety of pathways of tumor cytotoxicity different from cytotoxic chemotherapy have been proposed for this therapeutic **antibody** including **antibody**-dependent cellular cytotoxicity and complement-mediated cell lysis. This report describes that a proportion of patients with CLL receiving rituximab

treatment have in vivo activation of caspase-9, caspase-3, and poly(ADP-ribose) polymerase (PARP) cleavage in blood leukemia cells immediately following infusion of rituximab. This suggests that apoptosis using a pathway similar to fludarabine and other chemotherapeutic agents is intricately involved in the blood elimination of tumor cells after rituximab treatment. Patients having caspase-3 activation and PARP cleavage in vivo had a significantly lower blood leukemia cell count after treatment as compared to those without caspase activation. Significant down-modulation of the antiapoptotic proteins XIAP and Mcl-1 was also noted, possibly explaining in part how rituximab sensitizes CLL cells to the cytotoxic effect of chemotherapy in vivo. These findings suggest that the therapeutic benefit of **antibody**-based therapy in vivo for patients with CLL depends in part on induction of apoptosis and provides another area of focus for studying mechanisms of **antibody** -resistance in neoplastic cells.

L3 ANSWER 13 OF 38 MEDLINE on STN

2002058345 Document Number: 21630048. PubMed ID: 11756562. Suppression of Akt signaling induces Fas ligand expression: involvement of caspase and Jun kinase activation in Akt-mediated Fas ligand regulation. Suhara Toshimitsu; Kim Hyo-Soo; Kirshenbaum Lorrie A; Walsh Kenneth. (Division of Cardiovascular Research, St. Elizabeth's Medical Center of Boston, Massachusetts 02135, USA.) MOLECULAR AND CELLULAR BIOLOGY, (2002 Jan) 22 (2) 680-91. Journal code: 8109087. ISSN: 0270-7306. Pub. country: United States. Language: English.

AB Fas and Fas ligand (FasL) expression has been detected in chronic vascular lesions, and Fas-mediated apoptosis of vascular smooth muscle cells (VSMC) may influence the integrity of the atherosclerotic plaque. Here we report that FasL is not expressed by normal VSMC, but its expression is upregulated by stresses that induce apoptosis, including serum deprivation, exposure to the phosphatidylinositol 3-kinase (PI 3-kinase) inhibitor wortmannin, and ablation of Akt signaling. Conversely, constitutive activation of Akt signaling diminished FasL expression in VSMC cultures exposed to low-mitogen media or wortmannin. Under conditions of suppressed PI 3-kinase/Akt signaling, VSMC apoptosis was partially inhibited by treatment with neutralizing **antibody** against FasL. Suppression of Akt signaling increased the activity of c-Jun N-terminal kinase, and transduction of dominant-negative c-Jun inhibited FasL induction under these conditions. Diminished Akt signaling promoted the cleavage of caspase 3, and both caspase 3 cleavage and FasL induction were inhibited by transduction of dominant-negative caspase 9 or the caspase 8 inhibitor CrmA. Similarly, induction of FasL by the Akt-regulated forkhead transcription factor FKHRL1 was dependent upon caspase and c-Jun activation. Taken together, these results indicate that the sequential activation of caspase 3 and c-Jun participates in the induction of FasL under conditions of suppressed Akt signaling or FKHRL1 activation and that FasL participates in a positive-feedback loop to promote cell death under conditions of cellular stress.

L3 ANSWER 14 OF 38 MEDLINE on STN

2002670958 Document Number: 22318732. PubMed ID: 12432255. Downregulation of c-FLIP sensitizes DU145 prostate cancer cells to Fas-mediated apoptosis. Hyer Marc L; Sudarshan Sunil; Kim Youngsoo; Reed John C; Dong Jian-yun; Schwartz David A; Norris James S. (Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, South Carolina 29425, USA.) Cancer Biol Ther, (2002 Jul-Aug) 1 (4) 401-6. Journal code: 101137842. ISSN: 1538-4047. Pub. country: United States. Language: English.

AB Although DU145 prostate cancer cells are resistant to exogenously applied Fas agonist CH-11 (anti-Fas monoclonal **antibody**), Fas-resistance can be overcome using a FasL expressing adenovirus (AdGFPFasL(TET)) [Hyer et al., Molecular Therapy, 2000; 2:348-58 (ref.12)]. The purpose of this study was to try to understand why DU145 cells are resistant to CH-11 and determine the signaling pathway utilized by AdGFPFasL(TET) to induce apoptosis in these Fas-resistant cells. Using immunoblot analysis, we

show that AdGFPFasL(TET) is capable of initiating the classic Fas-mediated apoptotic pathway in DU145 cells, which includes activation of caspases-8, -3, -7, and -9, BID cleavage, cytochrome c release from mitochondria, and PARP cleavage. In contrast, CH-11 binds to Fas, but is unable to transmit the death signal beyond the plasma membrane suggesting a block at the DISC (death inducing signaling complex). The anti-apoptotic protein c-FLIP (cellular Flice-like inhibitory protein), which has been shown to inhibit Fas-mediated apoptosis at the DISC, was down-regulated following AdGFPFasL(TET) treatment prompting us to investigate its role in inhibiting CH-11-induced cell death. Using c-FLIP anti-sense oligonucleotides to down-regulate c-FLIP we sensitized DU145 cells to CH-11-induced apoptosis. These data suggest that c-FLIP may play a critical role in regulating Fas-mediated apoptosis in prostate cancer cells and that modulation of c-FLIP may enhance Fas signaling based therapies.

L3 ANSWER 15 OF 38 MEDLINE on STN

2002411066 Document Number: 22155376. PubMed ID: 12164932. Ganglioside loss promotes survival primarily by activating integrin-linked kinase/Akt without phosphoinositide 3-OH kinase signaling. Sun Ping; Wang Xiao-Qi; Lopatka Keith; Bangash Suleman; Paller Amy S. (Department of Pediatrics, Children's Memorial Institute for Education and Research, North-western University Medical School, 2300 Children's Plaza, Chicago, IL 60614, U.S.A.) JOURNAL OF INVESTIGATIVE DERMATOLOGY, (2002 Jul) 119 (1) 107-17. Journal code: 0426720. ISSN: 0022-202X. Pub. country: United States. Language: English.

AB Keratinocyte gangliosides influence cellular functions, including proliferation, adhesion, migration, and differentiation. The effects of endogenous depletion of membrane gangliosides by gene transfection of a human ganglioside-specific sialidase on cell survival were investigated. Ganglioside depletion promotes survival of the human keratinocyte-derived SCC12 cell line through upregulated phosphorylation of beta1 integrin, and increased phosphorylation and activity of integrin-linked kinase, protein kinase B/Akt, and Bad, with resultant inhibition of caspase-9 activation. Ganglioside deficiency also increases expression of cyclins D1 and E, promoting cell cycle progression from G1 phase to S phase. Inhibition of either protein kinase B/Akt or integrin-linked kinase activity renders the ganglioside-deficient cells susceptible to triggers of apoptosis. Both serine-473 and threonine-308 sites of protein kinase B/Akt show increased phosphorylation in ganglioside-deficient cells, but the cell survival correlates with increased phosphorylation of the serine-473 site of Akt, not with increased phosphorylation of the threonine-308 site. Consistently, blockade of ganglioside GT1b function activates integrin-linked kinase and only the serine-473 site of protein kinase B/Akt. In contrast, **antibody**-induced blockade of GM3 function increases only threonine-308 phosphorylation of ganglioside-deficient cells. Whereas blockade of phosphoinositide 3-OH kinase function suppresses threonine-308 phosphorylation, it neither inhibits serine-473 phosphorylation nor triggers apoptosis. These data suggest that ganglioside depletion modulates cell survival primarily through protein kinase B/Akt stimulation by a pathway that does not require phosphoinositide 3-OH kinase and epidermal growth factor receptor signaling.

L3 ANSWER 16 OF 38 MEDLINE on STN

2002128342 Document Number: 21852550. PubMed ID: 11865194. Activation of caspase-8 is critical for sensitivity to cytotoxic anti-Fas **antibody**-induced apoptosis in human ovarian cancer cells. Hayakawa A; Wu J; Kawamoto Y; Zhou Y W; Tanuma S; Nakashima I; Suzuki H. (Department of Equipment Center for Research and Education, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan.) APOPTOSIS, (2002 Apr) 7 (2) 107-13. Journal code: 9712129. ISSN: 1360-8185. Pub. country: United States. Language: English.

AB Two ovarian cancer cell lines named NOS4 and SKOV-3 have been shown to have different sensitivities to a cytotoxic anti-Fas **antibody**,

CH-11. Although both cell lines express Fas molecules on the cell surfaces at the same intensities, apoptosis is induced by CH-11 in NOS4 cells but not in SKOV-3 cells. In this study, the different apoptosis-sensitivities of these cells were assessed. Both cell lines express almost the same levels of FADD, RIP, c-FLIP, FAP-1, Bax, Bcl-2 and Bcl-XL. Evidence of caspase-8, caspase-9 and caspase-3 activation and of cleavage of PARP and Bid was obtained in NOS4 cells but not in SKOV-3 cells. When triggered by FasL protein, DNA fragmentation and caspase-8 activation were observed in SKOV-3 cells, though they were not as clear as in NOS4 cells. All the anti-Fas **antibody**-mediated signals for apoptosis induction in NOS4 cells were completely blocked by a caspase-8-specific inhibitor, Z-IETD-FMK. These results indicate that the different sensitivities to the anti-Fas **antibody** are solely dependent on the activation of caspase-8, which could be influenced by yet unknown qualitative or quantitative abnormalities in molecules involved in DISC formation.

L3 ANSWER 17 OF 38 MEDLINE on STN

2002001289 Document Number: 21621066. PubMed ID: 11751162. Cytokine regulation of human intestinal primary epithelial cell susceptibility to Fas-mediated apoptosis. Martin Carla A; Panja Asit. (Gastrointestinal Research Laboratory, Division of Gastroenterology Hepatology and Nutrition, Department of Medicine, Winthrop-University Hospital, Mineola, New York 11501, USA.) AMERICAN JOURNAL OF PHYSIOLOGY. GASTROINTESTINAL AND LIVER PHYSIOLOGY, (2002 Jan) 282 (1) G92-G104. Journal code: 100901227. ISSN: 0193-1857. Pub. country: United States. Language: English.

AB The regulatory mechanisms of nontransformed intestinal epithelial cell apoptosis have not been thoroughly investigated. We determined the susceptibility and mechanism of Fas-mediated apoptosis in nontransformed human intestinal epithelial cells (HIPEC) in the presence and absence of inflammatory cytokines. Despite ample expression of Fas, HIPEC were relatively insensitive to Fas-mediated apoptosis in that agonist anti-Fas **antibody** (CH11) induced a <25% increase in HIPEC apoptosis. Pretreatment of HIPEC with interferon (IFN)-gamma, but not tumor necrosis factor-alpha or granulocyte-macrophage colony-stimulating factor, significantly increased CH11-induced apoptosis of these cells without increasing Fas expression. Increased apoptosis correlated with increased caspase 3 activation but not expression of procaspase 3. Also, there was a significant delay in the onset of Fas-mediated apoptosis in HIPEC, which correlated with the generation of an activated caspase 3 p22/20 subunit. HIPEC required both initiator caspases 8 and 9 activity but expressed significantly less of the zymogen form of these caspases than did control cells. IFN-gamma-mediated sensitization of HIPEC occurred upstream of caspase 9 activation and correlated with a small increase in procaspase 8 expression (<1-fold increase) and a significant increase in expression of an intermediate form (p35) of caspase 4 (3.3-fold increase).

L3 ANSWER 18 OF 38 MEDLINE on STN

2002128351 Document Number: 21852548. PubMed ID: 11865192. Thrombocytopenia in an animal model of malaria is associated with an increased caspase-mediated death of thrombocytes. Piguet P F; Kan C D; Vesin C. (Department of Pathology, University of Geneva, CH 1211, Switzerland.. pierre.piguet@medecine.unige.ch) . APOPTOSIS, (2002 Apr) 7 (2) 91-8. Journal code: 9712129. ISSN: 1360-8185. Pub. country: United States. Language: English.

AB Infection of mice with Plasmodium Berghei Anka (PbA) leads to a thrombocytopenia, due to a reduced platelet life span, eventually associated with a syndrome of severe or cerebral malaria (CM). Thrombocytopenia was associated with an increase in the number of microparticles (mcp) in plasma. More than >60% of these mcp were of platelet origin, as seen by staining with an anti-platelet **antibody**. The thrombocytopenia and the amount of mcp were decreased in mice treated with anti CD40L mAb, suggesting that CD40L is the main effector of the thrombocytopenia. Caspase-1, -3, -6, -8, -9 were

activated in platelets from infected mice, as seen by the binding of labeled probes or the amount of pro-caspase-3. Treatment of infected mice with the caspases inhibitor ZVAD-fmk decreased the number of mcp and the thrombocytopenia, showing that platelet caspases are responsible for platelet fragmentation. In addition, the caspase inhibitor also caused a decrease in the mortality associated with CM, indicating a critical role of caspases in the expression of CM.

L3 ANSWER 19 OF 38 MEDLINE on STN

2002076720 Document Number: 21661698. PubMed ID: 11803376. Pre-processed caspase-9 contained in mitochondria participates in apoptosis. Costantini P; Bruey J-M; Castedo M; Metivier D; Loeffler M; Susin S A; Ravagnan L; Zamzami N; Garrido C; Kroemer Guido. (Centre National de la Recherche Scientifique, UMR1599, Institut Gustave Roussy, 39 rue Camille-Desmoulins, F-94805 Villejuif, France.) CELL DEATH AND DIFFERENTIATION, (2002 Jan) 9 (1) 82-8. Journal code: 9437445. ISSN: 1350-9047. Pub. country: England: United Kingdom. Language: English.

AB As shown here, mitochondria purified from different organs (liver, brain, kidney, spleen and heart) contain both pro-caspase-9 and the processed, mature form of caspase-9. Purified liver mitochondria release mature caspase-9 upon induction of permeability transition in vitro. This is accompanied by a discrete increase in the enzymatic cleavage of pro-caspase-9 substrates. We found that SHEP neuroblastoma cells constitutively contain pre-processed caspase-9 in their mitochondria, using a combination of subcellular fractionation and immunofluorescence with an **antibody** specific for the processed caspase. This is a cell type-specific phenomenon since HeLa cells mitochondria mainly contain pro-caspase-9 and comparatively little processed caspase-9. Upon introduction of apoptosis, mitochondrial pro-caspase-9 translocates to the cytosol and to the nucleus. This phenomenon is inhibited by transfection with Bcl-2. In synthesis, we report the unexpected finding that mitochondria can contain a pre-processed caspase isoform in non-apoptotic cells. Bcl-2-mediated regulation of mitochondrial membrane permeabilization may contribute to apoptosis control by preventing mitochondrial, pre-processed caspase-9 from interacting with its cytosolic activators.

L3 ANSWER 20 OF 38 MEDLINE on STN

2001673465 Document Number: 21576187. PubMed ID: 11571294. Tumor necrosis factor-alpha induces Bax-Bak interaction and apoptosis, which is inhibited by adenovirus E1B 19K. Sundararajan R; Cuconati A; Nelson D; White E. (Rutgers University, Piscataway, New Jersey 08854, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Nov 30) 276 (48) 45120-7. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Tumor necrosis factor (TNF)-alpha-mediated death signaling induces oligomerization of proapoptotic Bcl-2 family member Bax into a high molecular mass protein complex in mitochondrial membranes. Bax complex formation is associated with the release of cytochrome c, which propagates death signaling by acting as a cofactor for caspase-9 activation. The adenovirus Bcl-2 homologue E1B 19K blocks TNF-alpha-mediated apoptosis by preventing cytochrome c release, caspase-9 activation, and apoptosis of virus-infected cells. TNF-alpha induces E1B 19K-Bax interaction and inhibits Bax oligomerization. Oligomerized Bax may form a pore to release mitochondrial proteins, analogous to the homologous pore-forming domains of bacterial toxins. E1B 19K can also bind to proapoptotic Bak, but the functional significance is not known. TNF-alpha signaling induced Bak-Bax interaction and both Bak and Bax oligomerization. E1B 19K was constitutively in a complex with Bak, and blocked the Bak-Bax interaction and oligomerization of both. The TNF-alpha-mediated cytochrome c and Smac/DIABLO release from mitochondria was inhibited by E1B 19K expression in adenovirus-infected cells. Since either Bax or Bak is essential for death signaling by TNF-alpha, the interaction between E1B 19K and both Bak and Bax may be required to inhibit their cooperative or independent oligomerization to release proteins from mitochondria which promote caspase activation and cell death.

L3 ANSWER 21 OF 38 MEDLINE on STN

2001342984 Document Number: 21299121. PubMed ID: 11406544. Resveratrol induces extensive apoptosis by depolarizing mitochondrial membranes and activating caspase-9 in acute lymphoblastic leukemia cells. Dorrie J; Gerauer H; Wachter Y; Zunino S J. (The Chair of Genetics, Friedrich-Alexander University of Erlangen-Nurnberg, D91058 Erlangen, Germany.) CANCER RESEARCH, (2001 Jun 15) 61 (12) 4731-9. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB Resveratrol, a plant antibiotic, has been found to have anticancer activity and was recently reported to induce apoptosis in the myeloid leukemia line HL60 by the CD95-CD95 ligand pathway. However, many acute lymphoblastic leukemias (ALLs), particularly of B-lineage, are resistant to CD95-mediated apoptosis. Using leukemia lines derived from patients with pro-B t(4;11), pre-B, and T-cell ALL, we show in this report that resveratrol induces extensive apoptotic cell death not only in CD95-sensitive leukemia lines, but also in B-lineage leukemic cells that are resistant to CD95-signaling. Multiple dose treatments of the leukemic cells with 50 microM resveratrol resulted in >/=80% cell death with no statistically significant cytotoxicity against normal peripheral blood mononuclear cells under identical conditions. Resveratrol treatment did not increase CD95 expression or trigger sensitivity to CD95-mediated apoptosis in the ALL lines. Inhibition of CD95-signaling with a CD95-specific antagonistic **antibody** indicated that CD95-CD95 ligand interactions were not involved in initiating resveratrol-induced apoptosis. However, in each ALL line, resveratrol induced progressive loss of mitochondrial membrane potential as measured by the dual emission pattern of the mitochondria-selective dye JC-1. The broad spectrum caspase inhibitor benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone failed to block the depolarization of mitochondrial membranes induced by resveratrol, further indicating that resveratrol action was independent of upstream caspase-8 activation via receptor ligation. However, increases in caspase-9 activity ranged from 4- to 9-fold in the eight cell lines after treatment with resveratrol. Taken together, these results point to a general mechanism of apoptosis induction by resveratrol in ALL cells that involves a mitochondria/caspase-9-specific pathway for the activation of the caspase cascade and is independent of CD95-signaling.

L3 ANSWER 22 OF 38 MEDLINE on STN

2001347249 Document Number: 21303209. PubMed ID: 11410525. Preferential induction of apoptosis by interferon (IFN)-beta compared with IFN-alpha2: correlation with TRAIL/Apo2L induction in melanoma cell lines. Chawla-Sarkar M; Leaman D W; Borden E C. (Center for Drug Discovery and Development, Taussig Cancer Center and Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio 44195, USA.) CLINICAL CANCER RESEARCH, (2001 Jun) 7 (6) 1821-31. Journal code: 9502500. ISSN: 1078-0432. Pub. country: United States. Language: English.

AB On the basis of in vitro inhibition of tumor cell growth, IFNs have been generally considered to be antiproliferative proteins. To probe further the potential mechanisms of the antitumor effects of IFNs, we have assessed apoptosis in response to IFN-alpha2 and IFN-beta in cell lines of varied histologies, with a focus on melanomas. Many of the cell lines tested underwent apoptosis in response to IFN-beta, as assessed both by Annexin V and terminal deoxynucleotidyl transferase-mediated nick end labeling staining. In general, IFN-beta had greater growth inhibitory and proapoptotic effects than IFN-alpha2 on all cell lines. The melanoma cell line WM9, sensitive to growth inhibition by IFNs, had a greater degree of apoptosis than A375 melanoma cells, which were largely resistant to antigrowth effects of IFNs. IFN-beta-induced apoptosis was dependent on activation of the caspase cascade with cleavage of caspases 3, 8, and 9 and of the caspase 3 substrate, poly(ADP-ribose) polymerase. Caspase inhibitors benzyloxycarbonyl-Val-Ala-Asp-fluoromethyl keton or benzyloxycarbonyl-Asp-Glu-Val-Asp-fluoromethyl keton, inhibited IFN-beta-induced apoptosis. Other changes associated with apoptosis, including the movement of cytochrome c from mitochondria to cytoplasm and

DNA fragmentation, were also identified in response to IFN-beta. Apo2L ligand [tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)] was one of the early genes induced by IFN-beta in apoptosis-sensitive WM9 cells. Other sensitive melanoma cell lines had a similar IFN-beta-specific induction of TRAIL. Neutralizing **antibody** to TRAIL inhibited IFN-beta-induced apoptosis in WM9 cells. In resistant A375 cells, IFN-beta did not induce TRAIL/Apo2L expression. Thus, induction of TRAIL by IFNs in some tumor types may initiate the apoptotic cascade. This study offers another mechanism for the antitumor effects of IFNs.

L3 ANSWER 23 OF 38 MEDLINE on STN

2001416424 Document Number: 21358268. PubMed ID: 11465715. Regulation of CD95 (Fas/APO-1)-induced apoptosis in human chondrocytes. Kuhn K; Lotz M. (The Scripps Research Institute, La Jolla, California 92037, USA.) ARTHRITIS AND RHEUMATISM, (2001 Jul) 44 (7) 1644-53. Journal code: 0370605. ISSN: 0004-3591. Pub. country: United States. Language: English.

AB OBJECTIVE: To examine the role of nuclear factor kappaB (NF-kappaB) and caspases 3, 8, and 9 in CD95-mediated apoptosis of normal chondrocytes. METHODS: First-passage chondrocytes from normal human knee cartilage were stimulated with CD95 **antibody**, and cell death was determined by annexin V binding and by an enzyme-linked immunosorbent assay. Activation of caspases 3, 8, and 9 was measured by Western blotting, and their role in death signaling was evaluated using caspase-specific small peptide inhibitors. The influence of NF-kappaB was determined by electrophoretic mobility shift assay (EMSA) and proteasome inhibition-dependent blocking of the degradation of inhibitor of NF-kappaB. RESULTS: Low levels of NF-kappaB activity were detected by EMSA in unstimulated chondrocytes. NF-kappaB activity was increased in response to agonistic CD95 **antibody**. CD95 **antibody**-induced apoptosis was potentiated by the proteasome inhibitors MG-132 and PS1, and this was associated with a reduced nuclear translocation of NF-kappaB. Proteasome inhibitors also caused the induction of DNA fragmentation by tumor necrosis factor alpha. Procaspase 3 processing was enhanced by the proteasome inhibitor MG-132. Procaspase 8 was undetectable by immunoblotting in whole cell lysates of chondrocytes, but caspase 8 messenger RNA was detected by reverse transcription-polymerase chain reaction. Furthermore, apoptosis induced by CD95 stimulation and proteasome inhibitors was blocked by the caspase 8-specific inhibitor Ac-IETD-CHO. Processing of procaspase 9 was not observed, and inhibition of CD95-dependent cell death by the caspase 9 inhibitor Ac-LEHD-CHO was not significant. CONCLUSION: These results suggest that CD95-dependent cell death is enhanced by NF-kappaB inhibition at and/or downstream of caspase 8 activation and that caspase 9 activation is not involved in CD95-mediated apoptosis in chondrocytes.

L3 ANSWER 24 OF 38 MEDLINE on STN

2001226078 Document Number: 21102142. PubMed ID: 11171371. Segregation of nucleolar components coincides with caspase-3 activation in cisplatin-treated HeLa cells. Horky M; Wurzer G; Kotala V; Anton M; Vojtesek B; Vacha J; Wesierska-Gadek J. (Department of Pathological Physiology, Faculty of Medicine, Masaryk University, Komenskeho namesti 2, 662 43 Brno, Czech Republic.. mhorky@med.muni.cz) . JOURNAL OF CELL SCIENCE, (2001 Feb) 114 (Pt 4) 663-70. Journal code: 0052457. ISSN: 0021-9533. Pub. country: England: United Kingdom. Language: English.

AB We studied morphological changes of the nucleoli in HeLa cells treated with cisplatin and compared them with induction of markers of programmed cell death and TUNEL staining. We used different light microscopic nucleolar staining methods allowing us to visualize not only nucleolar proteins but also nucleolar RNA. Our results show predominantly compact, centrally localized nucleoli in intact control HeLa cells. In cisplatin-treated HeLa cells, we found an early onset of nucleolar segregation of proteins detected by argyrophilic nucleolar organizer regions and anti-nucleolar monoclonal **antibody** as well as an increased immunoreactivity for activated caspase-3 after 6 hours.

Staining with Toluidine Blue and Methyl-green Pyronine revealed segregated nucleoli 12 hours after the treatment with cisplatin. TUNEL positivity in cisplatin-treated HeLa cells was accompanied by the aggregation of the argyrophilic proteins in the central portion of nucleus, disappearance of nucleolar RNA and shrinkage of the nucleus after 24 hours. Monitoring of the biochemical changes by immunoblotting revealed that activation of distinct caspases and degradation of their downstream protein substrates is executed in two phases. During an early apoptotic stage beginning 4.5 hours post treatment an activation of caspase-9 and caspase-3 was observed. This was accompanied by proteolytic cleavage of poly(ADP-ribose) polymerase-1 (PARP-1). The caspase-9 activation seems to be mediated by recruitment by the activating factor Apaf-1 because the increased accumulation of Apaf-1 and cytochrome C in cytosol preceded the generation of mature caspase-9 form. A second phase of apoptosis occurring between 10 and 15 hours post treatment was characterized by degradation of other nucleolar and nuclear proteins such as nuclear lamins, topoisomerase I and B23. In conclusion, remarkable segregation of nucleolar argyrophilic proteins, nucleolar RNA and a simultaneous activation of the cascade of caspases markedly preceded the TUNEL positivity in cisplatin-treated HeLa cells thereby substantiating the hypothesis that the nucleolus is a preferred target for caspase-3-dependent proteolysis in cisplatin-treated HeLa cells.

L3 ANSWER 25 OF 38 MEDLINE on STN

2001675616 Document Number: 21562690. PubMed ID: 11696559. alpha-Toxin is a mediator of Staphylococcus aureus-induced cell death and activates caspases via the intrinsic death pathway independently of death receptor signaling. Bantel H; Sinha B; Domschke W; Peters G; Schulze-Osthoff K; Janicke R U. (Department of Immunology and Cell Biology, University of Munster, 48149 Munster, Germany.) JOURNAL OF CELL BIOLOGY, (2001 Nov 12) 155 (4) 637-48. Journal code: 0375356. ISSN: 0021-9525. Pub. country: United States. Language: English.

AB Infections with Staphylococcus aureus, a common inducer of septic and toxic shock, often result in tissue damage and death of various cell types. Although S. aureus was suggested to induce apoptosis, the underlying signal transduction pathways remained elusive. We show that caspase activation and DNA fragmentation were induced not only when Jurkat T cells were infected with intact bacteria, but also after treatment with supernatants of various S. aureus strains. We also demonstrate that S. aureus-induced cell death and caspase activation were mediated by alpha-toxin, a major cytotoxin of S. aureus, since both events were abrogated by two different anti-alpha-toxin **antibodies** and could not be induced with supernatants of an alpha-toxin-deficient S. aureus strain. Furthermore, alpha-toxin-induced caspase activation in CD95-resistant Jurkat sublines lacking CD95, Fas-activated death domain, or caspase-8 but not in cells stably expressing the antiapoptotic protein Bcl-2. Together with our finding that alpha-toxin induces cytochrome c release in intact cells and, interestingly, also from isolated mitochondria in a Bcl-2-controlled manner, our results demonstrate that S. aureus alpha-toxin triggers caspase activation via the intrinsic death pathway independently of death receptors. Hence, our findings clearly define a signaling pathway used in S. aureus-induced cytotoxicity and may provide a molecular rationale for future therapeutic interventions in bacterial infections.

L3 ANSWER 26 OF 38 MEDLINE on STN

2001360169 Document Number: 21315350. PubMed ID: 11423913. Inhibition of phosphatidylinositol-3 kinase/Akt or mitogen-activated protein kinase signaling sensitizes endothelial cells to TNF-alpha cytotoxicity. Zhang L; Himi T; Morita I; Murota S. (Department of Cellular Physiological Chemistry, Graduate School, Tokyo Medical and Dental University 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549, Japan.) CELL DEATH AND DIFFERENTIATION, (2001 May) 8 (5) 528-36. Journal code: 9437445. ISSN: 1350-9047. Pub. country: England: United Kingdom. Language: English.

AB Bovine carotid artery endothelial (BAE) cells are resistant to tumor

necrosis factor-alpha (TNF), like most other cells. We examined if mitogen-activated protein (MAP) kinase and phosphatidylinositol-3 (PI3) kinase/Akt pathways are involved in this effect. In BAE cells, TNF activates MAP kinase in a MAP kinase kinase 1 (MEK1) manner and Akt in PI3-kinase-dependent manner. Pretreatment with either the MEK1 inhibitor U0126 or PI3-kinase inhibitor LY294002 sensitized BAE cells to TNF-induced apoptosis. Neither U0126 nor LY294002 pretreatment affected TNF-induced activation of NF-kappaB, suggesting that the MAP kinase or PI3-kinase/Akt-mediated anti-apoptotic effect induced by TNF was not relevant to NF-kappaB activation. Both MAP kinase and PI3-kinase/Akt -mediated signaling could prevent cytochrome c release and mitochondrial transmembrane potential (Deltapsi) decrease. PI3-kinase/Akt signaling attenuated caspase-8 activity, whereas MAP kinase signaling impaired caspase-9 activity. These results suggest that TNF-induced MAP kinase and PI3-kinase/Akt signaling play important roles in protecting BAE cells from TNF cytotoxicity.

L3 ANSWER 27 OF 38 MEDLINE on STN

2001498604 Document Number: 21433667. PubMed ID: 11550089. Extended polyglutamine selectively interacts with caspase-8 and -10 in nuclear aggregates. U M; Miyashita T; Ohtsuka Y; Okamura-Oho Y; Shikama Y; Yamada M. (Department of Genetics, National Children's Medical Research Center, 3-35-31, Taishido, Setagaya, Tokyo 154-8509, Japan.) CELL DEATH AND DIFFERENTIATION, (2001 Apr) 8 (4) 377-86. Journal code: 9437445. ISSN: 1350-9047. Pub. country: England: United Kingdom. Language: English.

AB A growing number of inherited neurodegenerative disorders, including Huntington's disease, have been shown to be caused by the expansion of CAG/polyglutamine repeats. The molecular mechanism underlying these disorders, however, has yet to be clarified. We and others previously demonstrated that caspase-8 was activated by proteolysis in association with the expression of extended polyglutamine. Here, we further analyzed the selectivity of caspases in the process mediated by extended polyglutamine. Among upstream caspases, caspase-10, a close homolog of caspase-8, was also proteolytically activated, but caspase-9 was not. Caspase-8 and -10 were recruited into nuclear aggregates of extended polyglutamine, where at least a fraction of these caspases was converted to the activated forms. Caspase-8 and -10 were co-immunoprecipitated with polyglutamine only when the polyglutamine was pathologically extended, whereas caspase-2, -3, -6, -7 and -9 were not co-immunoprecipitated with polyglutamine regardless of its size. A dominant-negative form of caspase-8 with a mutation at the catalytic cysteine residue inhibited polyglutamine-mediated nuclear apoptotic phenotype. These results suggest that caspase-8 and -10 are autoactivated as a result of close proximity of the proforms of these molecules that occurs due to aggregate formation, which reveals a novel toxic gain-of-function mechanism for the pathogenesis of CAG-repeat disorders.

L3 ANSWER 28 OF 38 MEDLINE on STN

2001498600 Document Number: 21433663. PubMed ID: 11550085. Caspase-9 processing by caspase-3 via a feedback amplification loop in vivo. Fujita E; Egashira J; Urase K; Kuida K; Momoi T. (Division of Development and Differentiation, National Institute of Neuroscience, NCNP, Kodaira, Tokyo 187-8502, Japan.) CELL DEATH AND DIFFERENTIATION, (2001 Apr) 8 (4) 335-44. Journal code: 9437445. ISSN: 1350-9047. Pub. country: England: United Kingdom. Language: English.

AB In contrast to the autoprocessing of caspase-9, little is known about the biological significance of caspase-9 processing by caspase-3 via a feedback loop in vivo. We prepared antisera against mouse caspase-9 cleavage sites so that only the activated form of mouse caspase-9 was recognized. Using these antisera and caspase-9- and caspase-3-deficient mouse embryonic fibroblasts, we demonstrated that mouse caspase-9 is initially autoprocessed at D(353) and D(368) at low levels during staurosporine-induced apoptosis, whereupon the D(368) and D(168) sites are preferentially processed over D(353) by activated caspase-3 as part of a feedback amplification loop. Ac-DEVD-MCA (caspase-3-like) and Ac-LEHD-MCA

(caspase-9-like) cleavage activities clearly showed that caspase-9 autoprocessing was necessary for the activation of caspase-3, whereas full activation of caspase-3 and caspase-9 was achieved only through the feedback amplification loop. This feedback amplification loop also played a predominant role during programmed cell death of dorsal root ganglia neurons at mouse embryonic day 11.5.

L3 ANSWER 29 OF 38 MEDLINE on STN

2001323158 Document Number: 21137954. PubMed ID: 11238216. Butyric acid-induced T-cell apoptosis is mediated by caspase-8 and -9 activation in a Fas-independent manner. Kurita-Ochiai T; Ochiai K; Fukushima K. (Department of Microbiology, Nihon University School of Dentistry at Matsudo, Matsudo, Chiba 271-8587, Japan.. tkurita@mascat.nihon-u.ac.jp) . CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, (2001 Mar) 8 (2) 325-32. Journal code: 9421292. ISSN: 1071-412X. Pub. country: United States. Language: English.

AB Our previous study demonstrated that butyric acid, an extracellular metabolite of periodontopathic bacteria, induced apoptosis in murine thymocytes, splenic T cells, and human Jurkat cells. In this study, we examined whether CD95 ligand-receptor interaction is involved in butyric acid-induced T-cell apoptosis. Flow cytometry analysis indicated that expression of Fas in Jurkat and T cells from peripheral blood mononuclear cells was not affected by butyric acid treatment. Furthermore, the expression of Fas and FasL protein in Western blotting was not affected by butyric acid treatment. Coincubation with blocking anti-Fas **antibodies** prevented Fas-induced apoptosis but not butyric acid-induced apoptosis. Anti-FasL **antibodies** also did not prevent butyric acid-induced apoptosis at any dose examined. Although cytotoxic anti-Fas **antibody** affected butyric acid-induced apoptosis, a synergistic effect was not seen. Time-dependent activation of caspase-8 and -9 was recognized in butyric acid- as well as Fas-mediated apoptosis. IETD-CHO and LEHD-CHO, specific inhibitors of caspase-8 and -9, respectively, completely blocked Fas-mediated apoptosis and partially prevented butyric acid-induced apoptosis. These results suggest that the Fas-FasL interaction is not involved in butyric acid-induced apoptosis and that caspase-8 and -9-dependent apoptosis plays an important role in butyric acid-induced apoptosis, as well as Fas-induced apoptosis.

L3 ANSWER 30 OF 38 MEDLINE on STN

2000320204 Document Number: 20320204. PubMed ID: 10864208. Induction of apoptosis and activation of the caspase cascade by anti-EGF receptor monoclonal **antibodies** in DiFi human colon cancer cells do not involve the c-jun N-terminal kinase activity. Liu B; Fang M; Schmidt M; Lu Y; Mendelsohn J; Fan Z. (Department of Experimental Therapeutics, The University of Texas MD Anderson Cancer Center, Houston 77030, USA.) BRITISH JOURNAL OF CANCER, (2000 Jun) 82 (12) 1991-9. Journal code: 0370635. ISSN: 0007-0920. Pub. country: SCOTLAND: United Kingdom. Language: English.

AB We previously reported that exposure of DiFi human colon cancer cells to the anti-epidermal growth factor (EGF) receptor monoclonal **antibody** (mAb) 225 resulted in apoptosis, but the mechanisms remain to be elucidated. In the present study, we investigated the effects of a panel of four anti-EGF receptor mAbs, each of which binds to different epitopes of the EGF receptor in DiFi cells, on the induction of apoptosis. We found that each of these mAbs induced apoptosis in DiFi cells. Exposure of DiFi cells to mAb 225 activated the initiation caspase-8, which was detectable between 8 and 16 h after exposure of the cells to the **antibody**. There was also an activation of the initiation caspase-9, which lagged a few hours behind the activation of caspase-8. Exposure of DiFi cells to mAb 225 also activated the execution caspase-3, which was accompanied temporally by evidence of cleavage of a well-characterized caspase-3 substrate, poly(ADP)ribosepolymerase (PARP). Pre-exposure of the cells to the caspase-3-specific inhibitor DEVD-CHO partially reduced the mAb 225-induced PARP cleavage and apoptosis, whereas

pre-exposure of the cells to the caspase pan-inhibitor z-VAD-fmk completely inhibited mAb 225-induced apoptosis. Caspases-3, -8 and -9 were not activated in the cell lines in which mAb 225 only induced G1 phase arrest of the cell cycle. In contrast to the apoptosis of DiFi cells induced by ultraviolet irradiation, which strongly activated the c-jun N-terminal kinase-1 (JNK1) and the caspase cascade, mAb 225-induced apoptosis and activation of the caspase cascade in DiFi cells were not associated with activation of JNK1.

L3 ANSWER 31 OF 38 MEDLINE on STN
2000119360 Document Number: 20119360. PubMed ID: 10652256.
4-hydroxynonenal induces a cellular redox status-related activation of the caspase cascade for apoptotic cell death. Liu W; Kato M; Akhand A A; Hayakawa A; Suzuki H; Miyata T; Kurokawa K; Hotta Y; Ishikawa N; Nakashima I. (Department of Immunology, Nagoya University School of Medicine, Showa-ku, Nagoya 466-8550, Japan.) JOURNAL OF CELL SCIENCE, (2000 Feb) 113 (Pt 4) 635-41. Journal code: 0052457. ISSN: 0021-9533. Pub. country: ENGLAND: United Kingdom. Language: English.
AB 4-Hydroxynonenal (HNE), a diffusible product of lipid peroxidation, has been suggested to be a key mediator of oxidative stress-induced cell death. In this study, we partially characterized the mechanism of HNE-mediated cytotoxicity. Incubation of human T lymphoma Jurkat cells with 20-50 microM HNE led to cell death accompanied by DNA fragmentation. Western blot analysis showed that HNE-treatment induced time- and dose-dependent activation of caspase-8, caspase-9 and caspase-3. HNE-induced caspase-3 processing was confirmed by a flow cytometric demonstration of increased catalytic activity on the substrate peptide. HNE treatment also led to remarkable cleavage of poly(ADP-ribose) polymerase (PARP), which was prevented by pretreatment of cells with DEVD-FMK as a caspase-3 inhibitor. The HNE-mediated activation of caspases, cleavage of PARP and DNA fragmentation were blocked by antioxidants cysteine, N-acety-L-cysteine and dithiothreitol, but not by two other HNE-reactive amino acids lysine and histidine, or by cystine, the oxidized form of cysteine. HNE rapidly decreased levels of intracellular reduced glutathione (GSH) and its oxidized form GSSG, and these were also attenuated by the reductants. Coincubation of Jurkat cells with a blocking anti-Fas **antibody** prevented Fas-induced but not HNE-induced activation of caspase-3. HNE also activated caspase-3 in K562 cells that do not express functional Fas. Our results thereby demonstrate that HNE triggers oxidative stress-linked apoptotic cell death through activation of the caspase cascade. The results also suggest a possible mechanism involving a direct scavenge of intracellular GSH by HNE.

L3 ANSWER 32 OF 38 MEDLINE on STN
2000238091 Document Number: 20238091. PubMed ID: 10773825. Bcl-xL does not inhibit the function of Apaf-1. Newmeyer D D; Bossy-Wetzel E; Kluck R M; Wolf B B; Beere H M; Green D R. (La Jolla Institute for Allergy and Immunology, 10355 Science Center Road, San Diego, CA 92121, USA.) CELL DEATH AND DIFFERENTIATION, (2000 Apr) 7 (4) 402-7. Journal code: 9437445. ISSN: 1350-9047. Pub. country: ENGLAND: United Kingdom. Language: English.
AB Bcl-2 and its relative, Bcl-xL, inhibit apoptotic cell death primarily by controlling the activation of caspase proteases. Previous reports have suggested at least two distinct mechanisms: Bcl-2 and Bcl-xL may inhibit either the formation of the cytochrome c/Apaf-1/caspase-9 apoptosome complex (by preventing cytochrome c release from mitochondria) or the function of this apoptosome (through a direct interaction of Bcl-2 or Bcl-xL with Apaf-1). To evaluate this latter possibility, we added recombinant Bcl-xL protein to cell-free apoptotic systems derived from Jurkat cells and Xenopus eggs. At low concentrations (50 nM), Bcl-xL was able to block the release of cytochrome c from mitochondria. However, although Bcl-xL did associate with Apaf-1, it was unable to inhibit caspase activation induced by the addition of cytochrome c, even at much higher concentrations (1-5 microM). These observations, together with previous results obtained with Bcl-2, argue that Bcl-xL and Bcl-2 cannot

block the apoptosome-mediated activation of caspase-9.

L3 ANSWER 33 OF 38 MEDLINE on STN
2000072264 Document Number: 20072264. PubMed ID: 10606248.
bcl-X(S)-induced cell death in 3T3 cells does not require or induce caspase activation. Fridman J S; Benedict M A; Maybaum J. (Department of Pharmacology, University of Michigan Medical School, Ann Arbor 48109-0504, USA.) CANCER RESEARCH, (1999 Dec 1) 59 (23) 5999-6004. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.
AB Using a tetracycline-regulated expression system, we have shown that expression of bcl-X(s) is sufficient to induce acute cell death in 3T3 cells, and that the manner in which these cells die is both morphologically and biochemically different from Fas/CD95-induced apoptosis. bcl-X(s) expression causes loss of the inner mitochondrial membrane potential (deltapsim) but does not induce caspase activation. Loss of viability, as determined by mitochondrial function and ethidium bromide exclusion, was not inhibited by the broad-spectrum caspase inhibitor zVAD-fmk or by expression of a dominant negative caspase 9 (9DN). However, zVAD-fmk was efficacious in inhibiting cell death triggered by an activating anti-Fas/CD95 **antibody**. In addition, bcl-X(s) does not possess the 5th and 6th alpha-helices (thought to be the membrane-spanning domains in bcl-2, bcl-X(L), and bax) and, therefore, should not be able to form membrane channels, thus eliminating this possible mechanism of action. The finding that bcl-X(s) kills 3T3 cells without caspase activation, along with the absence of membrane spanning domains in bcl-X(s), may, therefore, represent a novel cell death pathway for the pro-death bcl-2 family members.

L3 ANSWER 34 OF 38 MEDLINE on STN
1999443470 Document Number: 99443470. PubMed ID: 10515447. Improved artificial death switches based on caspases and FADD. Fan L; Freeman K W; Khan T; Pham E; Spencer D M. (Department of Microbiology and Immunology, Baylor College of Medicine, Houston, TX 77030, USA.) HUMAN GENE THERAPY, (1999 Sep 20) 10 (14) 2273-85. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.
AB A number of "suicide genes" have been developed as safety switches for gene therapy vectors or as potential inducible cytotoxic agents for hyperproliferative disorders, such as cancer or restenosis. However, most of these approaches have relied on foreign proteins, such as HSV thymidine kinase, that primarily target rapidly dividing cells. In contrast, novel artificial death switches based on chemical inducers of dimerization (CIDs) and endogenous proapoptotic molecules function efficiently in both dividing and nondividing cells. In this approach, lipid-permeable, nontoxic CIDs are used to conditionally cross-link target proteins that are fused to CID-binding domains (CBDs), thus activating signaling cascades leading to apoptosis. In previous reports, CID-regulated Fas and caspases 1, 3, 8, and 9 were described. Since the maximum efficacy of these artificial death switches requires low basal and high specific activity, we have optimized these death switches for three parameters: (1) extent of oligomerization, (2) spacing between CBDs and target proteins, and (3) intracellular localization. We describe improved conditional Fas and caspase 1, 3, 8, and 9 alleles that function at subnanomolar levels of the CID AP1903 to trigger apoptosis. Further, we demonstrate for the first time that oligomerization of the death effector domain of the Fas-associated protein, FADD, is sufficient to trigger apoptosis, suggesting that the primary function of FADD, like that of Apaf-1, is oligomerization of associated caspases. Finally, we demonstrate that nuclear-targeted caspases 1, 3, and 8 can trigger apoptosis efficiently, implying that the cleavage of nuclear targets is sufficient for apoptosis.

L3 ANSWER 35 OF 38 MEDLINE on STN
1999310583 Document Number: 99310583. PubMed ID: 10381357. Activation of caspase-3 apoptotic pathways in skeletal muscle fibers in laminin alpha2-deficient mice. Mukasa T; Momoi T; Momoi M Y. (National Institute of Neuroscience, NCNP, Tokyo, Kodaira, 187-8502, Japan.) BIOCHEMICAL AND

BIOPHYSICAL RESEARCH COMMUNICATIONS, (1999 Jun 24) 260 (1) 139-42. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB dy/dy mice, which carry an unidentified mutation in the Lama2 gene, show dystrophic pathologies similar to those of human congenital muscular dystrophy. Laminin alpha2 deficiency induces apoptosis with DNA fragmentation. Caspases, which are involved in various types of cell death, are sequentially activated through a processing by other members of caspases. By using a cleavage site-directed **antibody** against caspase-3 that specifically reacts with the active form of caspase-3, we immunochemically demonstrated that caspase-3 is activated in the skeletal muscle fiber of dy/dy mice and that some of the activated caspase-3 muscle fibers are TUNEL-positive. Thus the lack of laminin alpha2 signals activates caspase-3, resulting in the apoptosis of muscle fibers.

L3 ANSWER 36 OF 38 MEDLINE on STN

1999255851 Document Number: 99255851. PubMed ID: 10320634. Functional absence of FADD in PLC/PRF/5 hepatoma cells: possible involvement in the transformation to hepatoma in HBV-infected hepatocytes. Suzuki A; Araki T; Miura M; Tsutomi Y. (Drug Safety Research Laboratory, Daiichi Pharmaceutical Co., Ltd., Tokyo 134, Japan.) PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, (1999 May) 221 (1) 72-9. Journal code: 7505892. ISSN: 0037-9727. Pub. country: United States. Language: English.

AB The death receptor Fas transduces apoptotic death signaling upon stimulation by Fas ligand and plays a key role in viral hepatitis. When hepatitis-B virus (HBV) infects hepatocytes, the Fas ligand/Fas system responds as the triggering machinery of hepatitis. However, some HBV-infected cells may circumvent Fas-mediated apoptosis and transform to hepatoma cells, as do PLC/PRF/5 hepatoma cells. Therefore, in the present study, we used PLC/PRF/5 hepatoma cells to investigate this ability to avoid Fas-mediated apoptosis. When the cells were treated with an agonistic Fas **antibody**, they showed resistance to Fas-mediated apoptosis. In contrast, HepG2 cells of the same hepatoma line succumbed. Caspase 3 and 8, which are essential regulators for Fas-mediated cell death, were expressed in both hepatoma cell lines, but only HepG2 cells showed activation of the caspases. A comparison study of expression of other death-associated factors between PLC/PRF/5 and HepG2 cells revealed no apparent differences. However, Far-Western blotting analysis using the Fas death domain (FDD) showed a significant difference. Molecular weight comparison and immunoblotting analysis revealed that PLC/PRF/5 cells lack the FDD-associated protein FADD. In addition, FDD-injected HepG2 cells showed a resistance to Fas-mediated apoptosis, and PLC/PRF/5 cells acquired Fas-sensitivity by FADD injection. Here, we propose that a functional absence of FADD is one of the pathways for the carcinogenesis of HBV-infected hepatocytes.

L3 ANSWER 37 OF 38 MEDLINE on STN

1998157986 Document Number: 98157986. PubMed ID: 9488720. Caspase-9, Bcl-XL, and Apaf-1 form a ternary complex. Pan G; O'Rourke K; Dixit V M. (Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Mar 6) 273 (10) 5841-5. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Genetic analysis of apoptosis in the nematode Caenorhabditis elegans has revealed the cell death machine to be composed of three core interacting components. CED-4 (equivalent to mammalian Apaf-1) is a nucleotide binding molecule that complexes with the zymogen form of the death protease CED-3, leading to its autoactivation and cell death. CED-9 blocks death by complexing with CED-4 and attenuating its ability to promote CED-3 activation. An equivalent ternary complex was found to be present in mammalian cells involving Apaf-1, the mammalian death protease caspase-9, and Bcl-XL, an anti-apoptotic member of the Bcl-2 family. Consistent with a central role for caspase-9, a dominant negative form

effectively inhibited cell death initiated by a wide variety of inducers.

L3 ANSWER 38 OF 38 CAPLUS COPYRIGHT 2003 ACS on STN
1997:761953 Document No. 128:31833 Cloning of human interleukin-1.beta. converting enzyme-like apoptotic protease-6 and its diagnostic and therapeutic applications. Dixit, Vishva M.; He, Wei-wu; Ruben, Steven M.; Kikly, Kristine K. (Smithkline Beecham Corp., USA; Human Genome Sciences, Inc.; University of Michigan). Eur. Pat. Appl. EP 808904 A2 19971126, 44 pp. DESIGNATED STATES: R: BE, CH, DE, DK, FR, GB, IT, LI, NL. (English). CODEN: EPXXDW. APPLICATION: EP 1997-303397 19970519. PRIORITY: US 1996-17949 19960520; US 1996-20344 19960523; US 1996-18961 19960605.

AB Members of the ICE/Ced-3 gene family are likely effector components of the cell death machinery. A novel member of this family designated ICE-LAP-6 is provided. By phylogenetic anal., **ICE-LAP6** is classified into the Ced-3 subfamily which includes Ced-3, Yama/CPP32/apopain, Mch2, and ICE-LAP3/Mch3/CMH-1. Interestingly, **ICE-LAP6** contains an active site QACGG pentapeptide, rather than the QACRG pentapeptide shared by other family members. Overexpression of **ICE-LAP6** induces apoptosis in MCF7 breast carcinoma cells. More importantly, **ICE-LAP6** is proteolytically processed into an active cysteine protease by granzyme B, an important component of cytotoxic T cell-mediated apoptosis. Once activated, **ICE-LAP6** is able to cleave the death substrate poly(ADP-ribose) polymerase into signature apoptotic fragments. Also disclosed are methods for utilizing such ICE LAP-6 for the treatment of a susceptibility to viral infection, tumorigenesis, and to diseases and defects in the control embryogenesis and tissue homeostasis, and the nucleic acid sequences described may be employed in an assay for ascertaining such susceptibility. Agonists and antagonists of ICE LAP-6 may also be used to treat various disease states.

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=> s l1 and CPP32
L4        1030 L1 AND CPP32

=> s l4 and monoclonal
L5         260 L4 AND MONOCLONAL

=> s l5 and polyclonal
L6          11 L5 AND POLYCLONAL

=> dup remove l6
PROCESSING COMPLETED FOR L6
L7            7 DUP REMOVE L6 (4 DUPLICATES REMOVED)

=> d l7 1-7 cbib abs
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L7 ANSWER 1 OF 7 MEDLINE on STN
2003332971 Document Number: 22747572. PubMed ID: 12864789. Immunoglobulin light chains modulate polymorphonuclear leucocyte apoptosis. Cohen G; Rudnicki M; Deicher R; Horl W H. (Department of Medicine III, University of Vienna, Vienna, Austria.. cohen@nephro.imed3.akh-wein.ac.at) . EUROPEAN JOURNAL OF CLINICAL INVESTIGATION, (2003 Aug) 33 (8) 669-76. Journal code: 0245331. ISSN: 0014-2972. Pub. country: England: United Kingdom. Language: English.

AB BACKGROUND: Apoptosis of polymorphonuclear leucocytes (PMNLs) is important for the resolution of inflammation. Recently, we demonstrated that glucose-modified proteins increase PMNL apoptosis. No protein factors in sera of uraemic patients attenuating PMNL apoptosis have been identified to date. MATERIALS AND METHODS: We tested the influence of commercially available **monoclonal** immunoglobulin light chains (IgLCs) from multiple myeloma patients and **polyclonal** IgLCs isolated from haemodialysis patients, previously shown to modulate PMNL functions and to contribute to their prestimulation, on PMNL apoptosis. We detected

morphological changes, DNA strand breaks and the loss of DNA content. RESULTS: All three apoptosis assays showed that kappa and lambda type IgLCs increase the percentage of viable PMNLs by inhibiting apoptosis in a concentration-dependent manner. The effect of IgLCs was abolished by specific **antibodies**. Addition of genistein abolished the reduction of PMNL apoptosis by IgLCs, suggesting that IgLCs exert their effect via tyrosine phosphorylation. Furthermore, we showed that the inhibition of caspase-3 activity is involved in the decrease of PMNL apoptosis. CONCLUSION: In concentrations present in sera of uraemic patients IgLCs could interfere with the normal resolution of inflammation and thereby contribute to the chronic inflammatory state found in end-stage renal disease patients.

L7 ANSWER 2 OF 7 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

2001:870986 The Genuine Article (R) Number: 484VR. Immunohistochemical analysis of apoptosis-related factors (Fas, Fas ligand, caspase-3 and single-stranded DNA) in ameloblastomas. Kumamoto H (Reprint); Kimi K; Ooya K. Tohoku Univ, Dept Oral Med & Bioregulat, Div Oral Pathol, Grad Sch Dent, Aoba Ku, 4-1 Seiryo Machi, Sendai, Miyagi 9808575, Japan (Reprint); Tohoku Univ, Dept Oral Med & Bioregulat, Div Oral Pathol, Grad Sch Dent, Aoba Ku, Sendai, Miyagi 9808575, Japan. JOURNAL OF ORAL PATHOLOGY & MEDICINE (NOV 2001) Vol. 30, No. 10, pp. 596-602. Publisher: MUNKSGAARD INT PUBL LTD. 35 NORRE SOGADE, PO BOX 2148, DK-1016 COPENHAGEN, DENMARK. ISSN: 0904-2512. Pub. country: Japan. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background: To clarify the possible role of apoptotic cell death in oncogenesis and cytodifferentiation of odontogenic epithelium, apoptosis-related factors - Fas, Fas ligand (FasL), caspase-3 and single-stranded DNA (ssDNA) - were analyzed in ameloblastomas as well as in tooth germs.

Methods: Specimens of 5 tooth germs, 29 benign ameloblastomas and 5 malignant ameloblastomas were examined by immunohistochemistry using anti-Fas, FasL, caspase-3 and ssDNA **polyclonal antibodies**.

Results: Immunoreactivity for Fas and FasL was detected in normal and neoplastic odontogenic epithelial cells. Fas expression in ameloblastomas was slightly lower than that in tooth germs, whereas FasL expression was similar in tooth germs and ameloblastomas. Malignant ameloblastomas showed downregulation of Fas expression and upregulation of FasL expression, as compared with benign ameloblastomas, indicating escape from cell death attack by immune cells. Immunoreactivity for caspase-3 was detected chiefly in cells neighboring the basement membrane in tooth germs and ameloblastomas. Expression of caspase-3 and Fas tended to be low in basal cell ameloblastomas and high in desmoplastic ameloblastomas, as compared with other variants of ameloblastomas. Caspase-3 expression was more intense in malignant ameloblastomas than in tooth germs and benign ameloblastomas. Apoptotic bodies reactive with anti-ssDNA **antibody** were detected in normal and neoplastic odontogenic epithelial cells detached from the basement membrane. Keratinizing cells in acanthomatous ameloblastomas and granular cells in granular cell ameloblastomas showed increased numbers of apoptotic bodies and increased expression of Fas and caspase-3, as compared with other neoplastic cells. Apoptotic reactions in malignant ameloblastomas were less frequent than in benign ameloblastomas, indicating abnormal regulation of cell turnover in odontogenic epithelial cells.

Conclusion: These apoptosis-related factors were detected in various patterns in normal and neoplastic odontogenic epithelium, suggesting that these factors might be associated with oncogenesis and cytodifferentiation of epithelial odontogenic tumors.

L7 ANSWER 3 OF 7 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

2001253064 EMBASE Blastocystis hominis: Evidence for caspase-3-like activity in cells undergoing programmed cell death. Nasirudeen A.M.A.; Singh M.; Yap E.H.; Tan K.S.W.. K.S.W. Tan, Department of Microbiology, Faculty of

Medicine, National University of Singapore, 5 Science Drive 2, Singapore 117597, Singapore. mictank@nus.edu.sg. Parasitology Research 87/7 (559-565) 2001.
Refs: 22.
ISSN: 0932-0113. CODEN: PARREZ. Pub. Country: Germany. Language: English. Summary Language: English.

AB We have shown previously that the human intestinal protozoan, Blastocystis hominis, undergoes apoptosis-like programmed cell death (PCD) when exposed to a cytotoxic **monoclonal antibody** (mAb), 1D5. In the present study, ELISA and immunoblot assays employing chicken anti-**CPP32 antibody** suggest that caspase-3-like antigens exist in B. hominis. Using colorimetric and flow cytometric assays for caspase-3 activity, we also observed an increase in activity between 1 h and 6 h after exposure to mAb 1D5, with greatest activity at 6 h. These findings suggest that caspase-3-like proteases play an important role in B. hominis undergoing PCD, similar to the phenomenon in higher eukaryotic organisms.

L7 ANSWER 4 OF 7 MEDLINE on STN

2001190064 Document Number: 21175844. PubMed ID: 11277999. Hypericin photo-induced apoptosis involves the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and activation of caspase-8. Schempp C M; Simon-Haarhaus B; Termeer C C; Simon J C. (Department of Dermatology, University of Freiburg, Hauptstrasse 7, D-79104, Freiburg, Germany.. schempp@haut.ukl.uni-freiburg.de) . FEBS LETTERS, (2001 Mar 23) 493 (1) 26-30. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB Hypericin (HYP) is a photosensitizing pigment from Hypericum perforatum that displays cytotoxic effects in neoplastic cell lines. Therefore, HYP is presently under consideration as a new anticancer drug in photodynamic therapy. Here, we investigated the mechanism of action of HYP photo-induced apoptosis of Jurkat cells compared to the cytostatic drug paclitaxel (PXL). Both photoactivated HYP and PXL similarly increased the activity of caspase-8 and caspase-3, and drug-induced apoptosis of Jurkat cells was completely blocked by inhibitors of caspase-8 (Z-IETD-FMK) and caspase-3 (Z-DEVD-FMK). The involvement of death receptors was analyzed using neutralizing **monoclonal antibodies** against Fas (SM1/23), FasL (NOK-2) and TNF-R1 (MAB225), and a **polyclonal** rabbit anti-human TNF-related apoptosis-inducing ligand (TRAIL) antiserum. TRAIL **antibody** blocked TRAIL-induced and HYP photo-induced, but not PXL-induced apoptosis of Jurkat cells. In contrast, PXL-induced, but not HYP-induced apoptosis was blocked by the SM1/23 and NOK-2 **antibodies**. Anti-TNF-R1 **antibody** had no effect. These findings suggest that HYP photo-induced apoptosis of Jurkat cells is mediated in part by the TRAIL/TRAIL-receptor system and subsequent activation of upstream caspases.

L7 ANSWER 5 OF 7 MEDLINE on STN

2001103761 Document Number: 20414355. PubMed ID: 10959797. Fluoride induces apoptosis by caspase-3 activation in human leukemia HL-60 cells. Anuradha C D; Kanno S; Hirano S. (Regional Environment Division, National Institute for Environmental Studies, Tsukuba, Ibaraki, Japan.) ARCHIVES OF TOXICOLOGY, (2000 Jul) 74 (4-5) 226-30. Journal code: 0417615. ISSN: 0340-5761. Pub. country: GERMANY: Germany, Federal Republic of. Language: English.

AB Even though fluoride toxicity is increasingly being considered to be important, very little information is available on the mechanism of action of fluoride. In the present study, the toxicity of fluoride on human leukemia (HL-60) cells was investigated and the involvement of caspase-3 was also studied. Fluoride induced apoptosis in HL-60 cells in a dose- and time-dependent manner. Annexin staining and DNA ladder formation on agarose gel electrophoresis further revealed that HL-60 cells underwent apoptosis on exposure to 2-5 mM fluoride. Western blotting using **polyclonal** anti-caspase-3 **antibody** and mouse anti-human poly(ADP-ribose) polymerase (PARP) **monoclonal antibody**

was performed to investigate caspase-3 and PARP activity. Fluoride led to the activation of caspase-3 which was evident by the loss of the 32 kDa precursor and appearance of the 17 kDa subunit. Furthermore, intact 116 kDa PARP was cleaved by fluoride treatment as shown by the appearance of a cleaved 89 kDa fragment. The results clearly suggest that fluoride causes cell death in HL-60 cells by causing the activation of caspase-3 which in turn cleaves PARP leading to DNA damage and ultimately cell death.

L7 ANSWER 6 OF 7 MEDLINE on STN

2000488775 Document Number: 20492868. PubMed ID: 11039724. Effects of transient global ischemia and kainate on poly(ADP-ribose) polymerase (PARP) gene expression and proteolytic cleavage in gerbil and rat brains. Liu J; Ying W; Massa S; Duriez P J; Swanson R A; Poirier G G; Sharp F R. (Department of Neurosurgery, University of California at San Francisco, 94121, USA.) BRAIN RESEARCH. MOLECULAR BRAIN RESEARCH, (2000 Aug 14) 80 (1) 7-16. Journal code: 8908640. ISSN: 0169-328X. Pub. country: Netherlands. Language: English.

AB Poly (ADP-ribose) polymerase (PARP) is involved in various cellular functions, including DNA repair, the cell cycle and cell death. While PARP activation could play a critical role in repairing ischemic brain damage, PARP inactivation caused by caspase 3-cleavage may also be important for apoptotic execution. In this study we investigated the effects of transient global ischemia and kainic acid (KA) neurotoxicity, in gerbil and rat brains, respectively, on PARP gene expression and protein cleavage. PARP mRNA increased in the dentate gyrus of gerbil brains 4 h after 10 min of global ischemia, which returned to basal levels 8 h after ischemia. KA injection (10 mg/kg) also induced a marked elevation in PARP mRNA level selectively in the dentate gyrus of rat brains 1 h following the injection, which returned to basal levels 4 h after the injection. These observations provide the first evidence of altered PARP gene expression in brains subjected to ischemic and excitotoxic insults. Using both **monoclonal** and **polyclonal antibodies** to PARP cleavage products, little evidence of significant PARP cleavage was found in gerbil brains within the first 3 days after 10 min of global ischemia. In addition, there was little evidence of significant PARP cleavage in rat brains within 2 days after kainate (KA) injection. Though these findings show that caspase induced PARP cleavage is not substantially activated by global ischemia and excitotoxicity in whole brain, the PARP mRNA induction could suggest a role for PARP in repairing DNA following brain injury.

L7 ANSWER 7 OF 7 MEDLINE on STN DUPLICATE 1

1999263380 Document Number: 99263380. PubMed ID: 10329597. Characterization of the interleukin-1beta-converting enzyme/ced-3-family protease, caspase-3/**CPP32**, in Hodgkin's disease: lack of caspase-3 expression in nodular lymphocyte predominance Hodgkin's disease. Izban K F; Wrone-Smith T; Hsi E D; Schnitzer B; Quevedo M E; Alkan S. (Department of Pathology and Cardinal Bernardin Cancer Center, Loyola University Medical Center, Maywood, Illinois, USA.) AMERICAN JOURNAL OF PATHOLOGY, (1999 May) 154 (5) 1439-47. Journal code: 0370502. ISSN: 0002-9440. Pub. country: United States. Language: English.

AB Apoptosis (programmed cell death) serves an important role in the normal morphogenesis, immunoregulation, and homeostatic mechanisms in both normal and neoplastic cells. Caspase-3/**CPP32**, a member of the ICE/Ced-3-family of cysteine proteases, is an important downstream mediator of several complex proteolytic cascades that result in apoptosis in both hematopoietic and nonhematopoietic cells. Previous studies have demonstrated that caspase-3 is commonly expressed in classical Hodgkin's disease (CHD); however, the biological significance of its expression in Hodgkin's disease is unknown. In this report, the expression of caspase-3 in nodular lymphocyte predominance Hodgkin's disease (NLPHD) was evaluated by immunohistochemistry; in addition, we investigated the role of caspase-3 in CD95 (Fas)-mediated apoptosis in three CHD cell lines. Formalin-fixed, paraffin-embedded tissue sections from 11 cases of NLPHD were immunostained for caspase-3 using a **polyclonal** rabbit

antibody that detects both the 32-kd zymogen and the 20-kd active subunit of the caspase-3 protease. Only 1/11 cases of NLPHD demonstrated caspase-3 immunopositivity in lymphocytic/histiocytic cells. Caspase-3 expression was also evaluated in three CHD cell lines, HS445, L428, and KMH2. Whereas caspase-3 expression was detected in HS445 and L428 cell lines, no expression was found in KMH2 cells by immunohistochemical staining. Treatment of HS445 and L428 cell lines for 72 hours with agonistic CD95 **monoclonal antibody** induced marked apoptosis that was significantly inhibited by pretreatment with the caspase-3 inhibitor, DEVD-FMK, as determined by terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling assay and flow cytometric analysis of 7-amino-actinomycin D staining. In addition, a significant increase in caspase-3 activity as determined by an enzyme colorimetric assay was detected in HS445 and L428 cells after 48 hours of CD95 stimulation. In marked contrast, treatment of caspase-3-deficient KMH2 cells with anti-CD95 mAb did not demonstrate an increase in caspase-3 activity or induce apoptosis. These data demonstrate caspase-3 is important for CD95-mediated apoptosis in CHD cell lines. In addition, the majority of NLPHD cases examined in this study failed to express detectable levels of caspase-3, suggesting these tumor cells may be resistant to apoptotic stimuli dependent on caspase-3 activity. Furthermore, these data suggest the differential expression of caspase-3 noted between NLPHD and CHD may provide additional evidence that each is a unique disease entity.

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     FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 09:14:11 ON
     20 OCT 2003
L1      2482251 S ANTIBOD?
L2           38 S L1 AND "ICE-LAP6"
L3           38 DUP REMOVE L2 (0 DUPLICATES REMOVED)
L4         1030 S L1 AND CPP32
L5          260 S L4 AND MONOCLONAL
L6           11 S L5 AND POLYCLONAL
L7            7 DUP REMOVE L6 (4 DUPLICATES REMOVED)

=> s l5 and chimeric
L8            4 L5 AND CHIMERIC

=> dup remove l8
PROCESSING COMPLETED FOR L8
L9            4 DUP REMOVE L8 (0 DUPLICATES REMOVED)

=> d l9 1-4 cbib abs

L9   ANSWER 1 OF 4     MEDLINE on STN
2003290164  Document Number: 22694353.    PubMed ID: 12810656.   Specific
     tumoricidal activity of a secreted proapoptotic protein consisting of HER2
     antibody and constitutively active caspase-3. Jia Lin-Tao; Zhang
     Li-Hong; Yu Cui-Juan; Zhao Jing; Xu Yan-Ming; Gui Jun-Hao; Jin Ming; Ji
     Zong-Ling; Wen Wei-Hong; Wang Cheng-Ji; Chen Si-Yi; Yang An-Gang.
     (Department of Biochemistry and Molecular Biology, Fourth Military Medical
     University, Xi'an 710032, China. ) CANCER RESEARCH, (2003 Jun 15) 63 (12)
     3257-62.  Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United
     States. Language: English.
AB   In this study, a novel approach to antitumor therapy was devised by
     generating a chimeric tumor-targeted killer protein, referred to
     as immunocasp-3, that comprises a single-chain anti-erbB2/HER2
     antibody with a NH(2)-terminal signal sequence, a Pseudomonas
     exotoxin A translocation domain, and a constitutively active caspase-3
     molecule.  In principle, cells transfected with the immunocasp-3 gene
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would express and secrete the **chimeric** protein, which then binds to HER2-overexpressing tumor cells. Subsequent cleavage of the constitutively active capase-3 domain from the immunocasp-3 molecule and its release from internalized vesicles would lead to apoptotic tumor cell death. To test this strategy, we transduced human lymphoma Jurkat cells with a **chimeric** immunocasp-3 gene expression vector and showed that they not only expressed and secreted the fusion protein but also selectively killed tumor cells overexpressing HER2 in vitro. i.v. injection of the transduced Jurkat cells led to tumor regression in a mouse xenograft model because of continuous secretion of immunocasp-3 by the transduced cells. The growth of HER2-positive tumor cells in this model was inhibited by i.m. as well as intratumor injection of immunocasp-3 expression plasmid DNA, indicating that the immunocasp-3 molecules secreted by transfected cells have systematic antitumor activity. We conclude that the immunocasp-3 molecule, combining the properties of a tumor-specific **antibody** with the proapoptotic activity of a caspase, has potent and selective antitumor activity, either as cell-based therapy or as a DNA vaccine. These findings provide a compelling rationale for therapeutic protocols designed for erbB2/HER2-positive tumors.

L9 ANSWER 2 OF 4 MEDLINE on STN

2003277702 Document Number: 22689106. PubMed ID: 12806611. Infliximab but not etanercept induces apoptosis in lamina propria T-lymphocytes from patients with Crohn's disease. Van den Brande Jan M H; Braat Henri; van den Brink Gijs R; Versteeg Henri H; Bauer Christiaan A; Hoedemaeker Inge; van Montfrans Catherine; Hommes Daan W; Peppelenbosch Maikel P; van Deventer Sander J H. (Laboratory for Experimental Internal Medicine, G2-133, Academic Medical Center, Meibergdreef 9, NL-1105 AZ Amsterdam, The Netherlands.. j.vandenbrande@amc.uva.nl) . GASTROENTEROLOGY, (2003 Jun) 124 (7) 1774-85. Journal code: 0374630. ISSN: 0016-5085. Pub. country: United States. Language: English.

AB BACKGROUND & AIMS: Steroid-refractory Crohn's disease responds to therapy with the **chimeric** anti-tumor necrosis factor (TNF)-alpha **antibody** infliximab. Etanercept, a recombinant TNF receptor/immunoglobulin G fusion protein, is highly effective in rheumatoid arthritis but not in Crohn's disease. Because both infliximab and etanercept are TNF-alpha-neutralizing drugs, we investigated the differences in TNF-alpha-neutralizing capacity and human lymphocyte binding and apoptosis-inducing capacity of both molecules. METHODS: We used a nuclear factor kappaB reporter assay and a cytotoxicity bioassay to study TNF-alpha neutralization by infliximab and etanercept. Lymphocyte binding and apoptosis-inducing capacity was investigated using fluorescence-activated cell sorter analysis, annexin V staining, and cleaved caspase-3 immunoblotting using mixed lymphocyte reaction-stimulated peripheral blood lymphocytes (PBL) from healthy volunteers and lamina propria T cells from patients with Crohn's disease. RESULTS: Both infliximab and etanercept neutralized TNF-alpha effectively. Infliximab bound to activated PBL and lamina propria T cells, whereas binding of etanercept was equal to a nonspecific control **antibody** . Infliximab but not etanercept induced peripheral and lamina propria lymphocyte apoptosis when compared with a control **antibody**. Infliximab activated caspase 3 in a time-dependent manner, whereas etanercept did not. CONCLUSIONS: Although both infliximab and etanercept showed powerful TNF-alpha neutralization, only infliximab was able to bind to PBL and lamina propria T cells and subsequently to induce apoptosis of activated lymphocytes. These data may provide a biological basis for the difference in efficacy of the 2 TNF-alpha-neutralizing drugs.

L9 ANSWER 3 OF 4 MEDLINE on STN

2002080583 Document Number: 21665829. PubMed ID: 11807010. The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. Byrd John C; Kitada Shinichi; Flinn Ian W; Aron Jennifer L; Pearson Michael; Lucas David; Reed John C. (Division of

Hematology-Oncology, The Ohio State University, B302 Starling Loving Hall, 320 W 10th Ave, Columbus, OH 43210, USA.. byrd-3@medctr.ohsu.edu) . BLOOD, (2002 Feb 1) 99 (3) 1038-43. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB *Rituximab is a* **chimeric monoclonal antibody** directed at CD20 with significant activity in non-Hodgkin lymphoma (NHL) and chronic lymphocytic leukemia (CLL). A variety of pathways of tumor cytotoxicity different from cytotoxic chemotherapy have been proposed for this therapeutic **antibody** including **antibody**-dependent cellular cytotoxicity and complement-mediated cell lysis. This report describes that a proportion of patients with CLL receiving rituximab treatment have in vivo activation of caspase-9, caspase-3, and poly(ADP-ribose) polymerase (PARP) cleavage in blood leukemia cells immediately following infusion of rituximab. This suggests that apoptosis using a pathway similar to fludarabine and other chemotherapeutic agents is intricately involved in the blood elimination of tumor cells after rituximab treatment. Patients having caspase-3 activation and PARP cleavage in vivo had a significantly lower blood leukemia cell count after treatment as compared to those without caspase activation. Significant down-modulation of the antiapoptotic proteins XIAP and Mcl-1 was also noted, possibly explaining in part how rituximab sensitizes CLL cells to the cytotoxic effect of chemotherapy in vivo. These findings suggest that the therapeutic benefit of **antibody**-based therapy in vivo for patients with CLL depends in part on induction of apoptosis and provides another area of focus for studying mechanisms of **antibody** -resistance in neoplastic cells.

L9 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2003 ACS on STN

2001:208111 Document No. 134:247241 Methods and compositions for modulating responsiveness to corticosteroids. Sekut, Les; Carter, Adam; Ghayur, Tariq; Banerjee, Subhashis; Tracey, Daniel E. (BASF A.-G., Germany). PCT Int. Appl. WO 2001019373 A2 20010322, 151 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG. (English). CODEN: PIXXD2. APPLICATION: WO 2000-US24725 20000908. PRIORITY: US 1999-398555 19990917.

AB Methods for modulating responsiveness to corticosteroids in a subject are provided. An agent which antagonizes a target that regulates prodn. of IFN-.gamma. in the subject is administered to the subject in combination with a corticosteroid such that responsiveness of the subject to the corticosteroid is modulated as compared to when a corticosteroid alone is administered to the subject. In one embodiment, the agent is an IL-18 antagonist. In another embodiment, the agent is an interleukin-12 (IL-12) antagonist. In yet another embodiment, the agent is an NK cell antagonist. In a preferred embodiment, the agent is an inhibitor of a caspase family protease, preferably an ICE inhibitor. In another preferred embodiment, the agent is an anti-IL-12 **monoclonal antibody**. In yet another preferred embodiment, the agent is an anti-asialo-GM1 **antibody** or an NK1.1 **antibody**. Other preferred agents include phosphodiesterase IV inhibitors and beta-2 agonists. The methods of the invention can be used in the treatment of a variety of inflammatory and immunol. diseases and disorders. Pharmaceutical compns. comprising an agent which antagonizes a target that regulates prodn. of IFN-.gamma. in a subject, a corticosteroid and a pharmaceutically acceptable carrier are also provided. A preferred compn. comprises an ICE inhibitor, a corticosteroid and a pharmaceutically acceptable carrier.

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(FILE 'HOME' ENTERED AT 09:13:56 ON 20 OCT 2003)

FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 09:14:11 ON 20 OCT 2003

L1 2482251 S ANTIBOD?
L2 38 S L1 AND "ICE-LAP6"
L3 38 *DUP REMOVE L2 (0 DUPLICATES REMOVED)*
L4 1030 S L1 AND CPP32
L5 260 S L4 AND MONOCLONAL
L6 11 S L5 AND POLYCLONAL
L7 7 DUP REMOVE L6 (4 DUPLICATES REMOVED)
L8 4 *S L5 AND CHIMERIC*
L9 4 DUP REMOVE L8 (0 DUPLICATES REMOVED)

=> s l3 and polyclonal
L10 1 L3 AND POLYCLONAL

=> d l10 cbib abs

L10 *ANSWER 1 OF 1* *MEDLINE on STN*
2003014489 Document Number: 22395753. PubMed ID: 12507932. Boswellic acids trigger apoptosis via a pathway dependent on caspase-8 activation but independent on Fas/Fas ligand interaction in colon cancer HT-29 cells. Liu Jian-Jun; Nilsson Ake; Oredsson Stina; Badmaev Vladimir; Zhao *Wan-Zhou; Duan Rui-Dong.* (Cell Biology B, Biomedical Center, B11, Lund University, Sweden.) CARCINOGENESIS, (2002 Dec) 23 (12) 2087-93. Journal code: 8008055. ISSN: 0143-3334. Pub. country: England: United Kingdom. Language: English.

AB Boswellic acids are the effective components of gum resin of Boswellia serrata, which has anti-inflammatory properties. Recent studies on brain tumors and leukemic cells indicate that boswellic acids may have antiproliferative and apoptotic effects with the mechanisms being not studied in detail. *We studied their antiproliferative and apoptotic* effects on colon cancer cells and the pathway leading to apoptosis. HT-29 cells were treated with beta-boswellic acid (BA), keto-beta-boswellic acid (K-BA) and acetyl-keto-beta-boswellic acid (AK-BA), respectively. Apoptosis was determined by flow cytometry, by cytoplasmic DNA-histone *complex and the activity* of caspase-3. The cleavage of poly-(ADP-ribose)-polymerase (PARP) and expression of Fas were examined by western blot. Specific caspase inhibitors, **polyclonal** Fas **antibody**, and antagonistic Fas **antibody** *ZB4* were employed to elucidate apoptotic pathways. DNA synthesis and cell viability were examined. Both K-BA and AK-BA increased cytoplasmic DNA-histone complex dose-dependently and increased pre-G(1) peak in flow cytometer analysis, with the effects of AK-BA being stronger than K-BA. *BA only increased the formation of DNA-histone complex at a high* concentration. K-BA and AK-BA increased caspase-8, caspase-9 and caspase-3 activities accompanied by cleavage of PARP. The effects of AK-BA on formation of cytoplasmic DNA histone and on caspase-3 activation were 3.7- and 3.4-fold, respectively, more effective than those induced by *camptothecin.* The apoptosis induced by AK-BA was inhibited completely by caspase-3 or caspase-8 inhibitor and partially by caspase-9 inhibitor. ZB4 blocked exogenous Fas ligand-induced apoptosis, but had no effect on AK-BA-induced apoptosis. AK-BA had no *significant effect on expression of* Fas. Apart from apoptotic effect, these acids also inhibited [(3)H]thymidine incorporation and cell viability to different extent. In conclusion, boswellic acids, particularly AK-BA and K-BA have antiproliferative and apoptotic effects in human HT-29 cells. The *apoptotic effect is mediated via a pathway dependent on caspase-8* activation but independent of Fas/FasL interaction.

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L11 4 "ICE-LAP 6"

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PROCESSING COMPLETED FOR L11
L12 4 DUP REMOVE L11 (0 DUPLICATES REMOVED)

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L12 ANSWER 1 OF 4 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
2001:563673 Document No.: PREV200100563673. Interleukin-1 beta converting enzyme like apoptotic protease-6. Dixit, Vishva M. [Inventor]; He, Wei-Wu [Inventor]; Kikly, Kristine K. [Inventor]; Ruben, Steven M. [Inventor]. ASSIGNEE: Human Genome Sciences, Inc.; SmithKline Beecham Corporation; University of Michigan. Patent Info.: US 6294169 September 25, 2001. Official Gazette of the United States Patent and Trademark Office Patents, (Sep. 25, 2001) Vol. 1250, No. 4. e-file.
CODEN: OGUPE7. ISSN: 0098-1133. Language: English.

AB Human **ICE LAP-6** polypeptides and DNA (RNA) encoding such **ICE LAP-6** and a procedure for producing such polypeptides by recombinant techniques is disclosed. Also disclosed are methods for utilizing such **ICE LAP-6** for the treatment of a susceptibility to viral infection, tumorogenesis and to diseases and defects in the control embryogenesis and tissue homeostasis, and the nucleic acid sequences described above may be employed in an assay for ascertaining such susceptibility. Antagonists against such **ICE LAP-6** and their use as a therapeutic to treat Alzheimer's disease, Parkinson's disease, rheumatoid arthritis, septic shock, sepsis, stroke, chronic inflammation, acute inflammation, CNS inflammation, osteoporosis, ischemia reperfusion injury, cell death associated with cardiovascular disease, polycystic kidney disease, apoptosis of endothelial cells in cardiovascular disease, degenerative liver disease, MS, ALS, cererbellar degeneration, ischemic injury, myocardial infarction, AIDS, myelodysplastic syndromes, aplastic anemia, male pattern baldness, and head injury damage are also disclosed. Also disclosed are diagnostic assays for detecting diseases related to mutations in the nucleic acid sequences and altered concentrations of the polypeptides. Also disclosed are diagnostic assays for detecting mutations in the polynucleotides encoding the interleukin-1 beta converting enzyme apoptosis proteases and for detecting altered levels of the polypeptide in a host.

L12 ANSWER 2 OF 4 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
2000:305066 Document No.: PREV200000305066. Interleukin-1 beta converting enzyme like apoptotic protease-6. Dixit, Vishva M. [Inventor, Reprint author]; He, Wei-Wu [Inventor]; Kikly, Kristine K. [Inventor]; Ruben, Steven M. [Inventor]. AnnArbor, MI, USA. ASSIGNEE: Smithkline Beecham Corporation. Patent Info.: US 6010878 January 04, 2000. Official Gazette of the United States Patent and Trademark Office Patents, (Jan. 4, 2000) Vol. 1230, No. 1. e-file.
CODEN: OGUPE7. ISSN: 0098-1133. Language: English.

AB Human **ICE LAP-6** polypeptides and DNA (RNA) encoding such **ICE LAP-6** and a procedure for producing such polypeptides by recombinant techniques is disclosed. Also disclosed are methods for utilizing such **ICE LAP-6** for the treatment of a susceptibility to viral infection, tumorogenesis and to diseases and defects in the control embryogenesis and tissue homeostasis, and the nucleic acid sequences described above may be employed in an assay for ascertaining such susceptibility. Antagonists against such **ICE LAP-6** and their use as a therapeutic to treat Alzheimer's disease, Parkinson's disease, rheumatoid arthritis, septic shock, sepsis, stroke, chronic inflammation, acute inflammation, CNS inflammation, osteoporosis, ischemia reperfusion injury, cell death associated with cardiovascular disease, polycystic kidney disease, apoptosis of endothelial cells in cardiovascular disease, degenerative liver disease, MS, ALS, cererbellar degeneration, ischemic injury, myocardial infarction, AIDS, myelodysplastic syndromes, aplastic

anemia, male pattern baldness, and head injury damage are also disclosed. Also disclosed are diagnostic assays for detecting diseases related to mutations in the nucleic acid sequences and altered concentrations of the polypeptides. Also disclosed are diagnostic assays for detecting mutations in the polynucleotides encoding the interleukin-1 beta converting enzyme apoptosis proteases and for detecting altered levels of the polypeptide in a host.

L12 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2003 ACS on STN
1997:761953 Document No. 128:31833 Cloning of human interleukin-1.beta. converting enzyme-like apoptotic protease-6 and its diagnostic and therapeutic applications. Dixit, Vishva M.; He, Wei-wu; Ruben, Steven M.; Kikly, Kristine K. (Smithkline Beecham Corp., USA; Human Genome Sciences, Inc.; University of Michigan). Eur. Pat. Appl. EP 808904 A2 19971126, 44 pp. DESIGNATED STATES: R: BE, CH, DE, DK, FR, GB, IT, LI, NL. (English). CODEN: EPXXDW. APPLICATION: EP 1997-303397 19970519. PRIORITY: US 1996-17949 19960520; US 1996-20344 19960523; US 1996-18961 19960605.

AB Members of the ICE/Ced-3 gene family are likely effector components of the cell death machinery. A novel member of this family designated **ICE-LAP-6** is provided. By phylogenetic anal., ICE-LAP6 is classified into the Ced-3 subfamily which includes Ced-3, Yama/CPP32/apopain, Mch2, and ICE-LAP3/Mch3/CMH-1. Interestingly, ICE-LAP6 contains an active site QACGG pentapeptide, rather than the QACRG pentapeptide shared by other family members. Overexpression of ICE-LAP6 induces apoptosis in MCF7 breast carcinoma cells. More importantly, ICE-LAP6 is proteolytically processed into an active cysteine protease by granzyme B, an important component of cytotoxic T cell-mediated apoptosis. Once activated, ICE-LAP6 is able to cleave the death substrate poly(ADP-ribose) polymerase into signature apoptotic fragments. Also disclosed are methods for utilizing such **ICE LAP-6** for the treatment of a susceptibility to viral infection, tumorigenesis, and to diseases and defects in the control embryogenesis and tissue homeostasis, and the nucleic acid sequences described may be employed in an assay for ascertaining such susceptibility. Agonists and antagonists of **ICE LAP-6** may also be used to treat various disease states.

L12 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2003 ACS on STN
1996:424018 Document No. 125:161742 ICE-LAP6, a novel member of the ICE/Ced-3 gene family, is activated by the cytotoxic T cell protease granzyme B. Duan, Hangjun; Orth, Kim; Chinnaiyan, Arul M.; Poirier, Guy G.; Froelich, Christopher J.; He, Wei-Wu; Dixit, Vishva M. (Dep. Pathology, Univ. Michigan Medical School, Ann Arbor, MI, 48109, USA). Journal of Biological Chemistry, 271(28), 16720-16724 (English) 1996. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB Members of the ICE/Ced-3 gene family are likely effector components of the cell death machinery. Here, we characterize a novel member of this family designated **ICE-LAP-6**. By phylogenetic anal., ICE-LAP6 is classified into the Ced-3 subfamily which includes Ced-3, Yama/CPP32/apopain, Mch2, and ICE-LAP3/Mch3/CMH-1. Interestingly, ICE-LAP6 contains an active site QACGG pentapeptide, rather than the QACRG pentapeptide shared by other family members. Overexpression of ICE-LAP6 induces apoptosis in MCF7 breast carcinoma cells. More importantly, ICE-LAP6 is proteolytically processed into an active cysteine protease by granzyme B, an important component of cytotoxic T cell-mediated apoptosis. Once activated, ICE-LAP6 is able to cleave the death substrate poly(ADP-ribose) polymerase into signature apoptotic fragments.

=> s (dixit v?/au or he w?/au or kikly k?/au)
L13 6101 (DIXIT V?/AU OR HE W?/AU OR KIKLY K?/AU)

=> s l13 and antibody

L14 486 L13 AND ANTIBODY

=> s l14 and ICE
L15 19 L14 AND ICE

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PROCESSING COMPLETED FOR L15
L16 10 DUP REMOVE L15 (9 DUPLICATES REMOVED)

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L16 ANSWER 1 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN
1998:653766 Document No. 129:256027 Cloning, sequence, and therapeutic use of human interleukin-1 .beta.-converting enzyme apoptosis protease-10 gene and enzyme. **Kikly, Kristine Kay** (SMITHKLINE BEECHAM CORPORATION, USA). Eur. Pat. Appl. EP 867513 A2 19980930, 19 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (English). CODEN: EPXXDW. APPLICATION: EP 1998-302150 19980323. PRIORITY: US 1997-42030 19970327.
AB Interleukin-1 .beta.-converting enzyme apoptosis protease-10 (**ICE**-LAP-10) polypeptides and polynucleotides and methods for producing such polypeptides by recombinant techniques are disclosed. Also disclosed are methods for utilizing **ICE**-LAP-10 polypeptides and polynucleotides in therapy, and diagnostic assays for such. The title gene and protein may be useful in treatment of cancer, inflammation, autoimmune disorders, allergy, asthma, rheumatoid arthritis, CNS inflammation, cerebellar degeneration, Alzheimer's disease, Parkinson's disease and multiple sclerosis. Other diseases include amyotrophic lateral sclerosis, head injury, septic shock sepsis, stroke, osteoporosis, osteoarthritis, ischemia reperfusion injury, cardiovascular disease, kidney disease, liver disease, ischemic injury, and myocardial infarction. Agonists, antagonists, ligands, receptors, **antibodies**, and vaccines specific for the title compd. are also claimed.

L16 ANSWER 2 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN
1998:314520 Document No. 129:25084 Human FIN-1 caspase sequences and uses in identification and treatment of tumors, viral infection, and other diseases. **Kikly, Kristine**; Emery, John G. (Smithkline Beecham Corp., USA). Eur. Pat. Appl. EP 841399 A2 19980513, 48 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI. (English). CODEN: EPXXDW. APPLICATION: EP 1997-309003 19971110. PRIORITY: US 1996-748086 19961112.
AB Human FIN-1 proteins and nucleic acid sequences encoding such proteins, methods for recombinant prodn. of FIN-1, its use in treatment of viral infections and cancer, and diagnostic assays relating to FIN-1 nucleic acid mutations and altered FIN-1 concn. are claimed. Factors which induce apoptosis can serve therapeutic purposes as antiviral agents, antitumor agents, or to control development and tissue homeostasis. Likewises, factors which prevent apoptosis can serve therapeutic purposes in treating ischemic injury such as stroke, myocardial infarction and reperfusion injury, for treating neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis; osteoporosis and osteoarthritis, polycystic kidney disease, chronic degenerative liver disease, AIDS, and aplastic anemia. FIN-1 (FLICE inhibitor-1), a caspase, inhibits the FLICE (**ICE**-LAP7) protease, an enzyme involved in the induction of apoptosis. Therefore, a mutation in the FIN-1 gene may be indicative of a susceptibility to viral diseases and tumors, and detection of mutated forms or altered expression can provide diagnostic tools. Similarly, FIN-1 sequences can be used for development of **antibodi s**, hybridization probes, inhibitors, and all manner of mols. suitable for identification and gene therapy of dysfunctions assocd. with the FLICE apoptotic mechanism.

L16 ANSWER 3 OF 10 MEDLINE on STN
1998157986 Document Number: 98157986. PubMed ID: 9488720. Caspase-9,

Bcl-XL, and Apaf-1 form a ternary complex. Pan G; O'Rourke K; **Dixit V M.** (Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Mar 6) 273 (10) 5841-5. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Genetic analysis of apoptosis in the nematode Caenorhabditis elegans has revealed the cell death machine to be composed of three core interacting components. CED-4 (equivalent to mammalian Apaf-1) is a nucleotide binding molecule that complexes with the zymogen form of the death protease CED-3, leading to its autoactivation and cell death. CED-9 blocks death by complexing with CED-4 and attenuating its ability to promote CED-3 activation. An equivalent ternary complex was found to be present in mammalian cells involving Apaf-1, the mammalian death protease caspase-9, and Bcl-XL, an anti-apoptotic member of the Bcl-2 family. Consistent with a central role for caspase-9, a dominant negative form effectively inhibited cell death initiated by a wide variety of inducers.

L16 ANSWER 4 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN

1997:761953 Document No. 128:31833 Cloning of human interleukin-1.beta. converting enzyme-like apoptotic protease-6 and its diagnostic and therapeutic applications. **Dixit, Vishva M.**; **He, Wei-wu** ; Ruben, Steven M.; **Kikly, Kristine K.** (Smithkline Beecham Corp., USA; Human Genome Sciences, Inc.; University of Michigan). Eur. Pat. Appl. EP 808904 A2 19971126, 44 pp. DESIGNATED STATES: R: BE, CH, DE, DK, FR, GB, IT, LI, NL. (English). CODEN: EPXXDW. APPLICATION: EP 1997-303397 19970519. PRIORITY: US 1996-17949 19960520; US 1996-20344 19960523; US 1996-18961 19960605.

AB Members of the **ICE**/Ced-3 gene family are likely effector components of the cell death machinery. A novel member of this family designated **ICE**-LAP-6 is provided. By phylogenetic anal., **ICE**-LAP6 is classified into the Ced-3 subfamily which includes Ced-3, Yama/CPP32/apopain, Mch2, and **ICE**-LAP3/Mch3/CMH-1. Interestingly, **ICE**-LAP6 contains an active site QACGG pentapeptide, rather than the QACRG pentapeptide shared by other family members. Overexpression of **ICE**-LAP6 induces apoptosis in MCF7 breast carcinoma cells. More importantly, **ICE**-LAP6 is proteolytically processed into an active cysteine protease by granzyme B, an important component of cytotoxic T cell-mediated apoptosis. Once activated, **ICE**-LAP6 is able to cleave the death substrate poly(ADP-ribose) polymerase into signature apoptotic fragments. Also disclosed are methods for utilizing such **ICE** LAP-6 for the treatment of a susceptibility to viral infection, tumorigenesis, and to diseases and defects in the control embryogenesis and tissue homeostasis, and the nucleic acid sequences described may be employed in an assay for ascertaining such susceptibility. Agonists and antagonists of **ICE** LAP-6 may also be used to treat various disease states.

L16 ANSWER 5 OF 10 CAPLUS COPYRIGHT 2003 ACS on STN

1997:761853 Document No. 128:31110 Cloning and cDNA sequence of human interleukin-1.beta. converting enzyme-like apoptotic protease 7. **Dixit, Vishva M.**; **Kikly, Kristine K.**; Ni, Jian; Rosen, Craig A.; Ruben, Steven M. (Smithkline Beecham Corp., USA; Human Genome Sciences, Inc.; University of Michigan). Eur. Pat. Appl. EP 807686 A2 19971119, 48 pp. DESIGNATED STATES: R: BE, CH, DE, DK, FR, GB, IT, LI, NL. (English). CODEN: EPXXDW. APPLICATION: EP 1997-303203 19970512. PRIORITY: US 1996-17914 19960516; US 1996-17454 19960517; US 1996-19365 19960605.

AB Human interleukin-1.beta. converting enzyme apoptosis protease-7 (**ICE** LAP-7, or FLICE) polypeptides and DNA (RNA) encoding such **ICE** LAP-7 and a procedure for producing such polypeptides by recombinant techniques is disclosed. Also disclosed are methods for utilizing such **ICE** LAP-7 for the treatment of a susceptibility to viral infection, tumorigenesis, and to diseases and defects in the control embryogenesis and tissue homeostasis, and the nucleic acid sequences described above may be employed in an assay for ascertaining

such susceptibility. Antagonists against such **ICE** LAP-7 and their use as a therapeutic to treat Alzheimer's disease, Parkinson's disease, rheumatoid arthritis, septic shock, sepsis, stroke, chronic inflammation, acute inflammation, CNS inflammation, osteoporosis, ischemia reperfusion injury, cell death assocd. with cardiovascular disease, polycystic kidney disease, apoptosis of endothelial cells in cardiovascular disease, degenerative liver disease, MS, ALS, cerebellar degeneration, ischemic injury, myocardial infarction, AIDS, myelodysplastic syndromes, aplastic anemia, male pattern baldness, and head injury damage are also disclosed. Also disclosed are diagnostic assays for detecting diseases related to mutations in the nucleic acid sequences and altered concns. of the polypeptides. Also disclosed are diagnostic assays for detecting mutations in the polynucleotides encoding the interleukin-1.beta. converting enzyme apoptosis proteases and for detecting altered levels of the polypeptide in a host.

L16 ANSWER 6 OF 10 MEDLINE on STN DUPLICATE 1

97160607 Document Number: 97160607. PubMed ID: 9006941. FLICE induced apoptosis in a cell-free system. Cleavage of caspase zymogens. Muzio M; Salvesen G S; **Dixit V M**. (Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 Jan 31) 272 (5) 2952-6. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Engagement of CD95 or tumor necrosis factor 1 receptor (TNFR-1) by ligand or agonist **antibodies** is capable of activating the cell death program, the effector arm of which is composed of mammalian interleukin-1beta converting enzyme (**ICE**)-like cysteine proteases (designated caspases) that are related to the Caenorhabditis elegans death gene, CED-3. Caspases, unlike other mammalian cysteine proteases, cleave their substrates following aspartate residues. Furthermore, proteases belonging to this family exist as zymogens that in turn require cleavage at internal aspartate residues to generate the two-subunit active enzyme. As such, family members are capable of activating each other. Remarkably, both CD95 and TNFR-1 death receptors initiate apoptosis by recruiting a novel **ICE**/CED-3 family member, designated FLICE/MACH, to the receptor signaling complex. Therefore, FLICE/MACH represents the apical triggering protease in the cascade. Consistent with this, recombinant FLICE was found capable of proteolytically activating downstream caspases. Furthermore, CrmA, a pox virus-encoded serpin that inhibits Fas and tumor necrosis factor-induced cell death attenuates the ability of FLICE to activate downstream caspases.

L16 ANSWER 7 OF 10 MEDLINE on STN

96214865 Document Number: 96214865. PubMed ID: 8617712. Molecular ordering of the cell death pathway. Bcl-2 and Bcl-xL function upstream of the CED-3-like apoptotic proteases. Chinnaiyan A M; Orth K; O'Rourke K; Duan H; Poirier G G; **Dixit V M**. (Department of Pathology, University of Michigan Medical School, Ann Arbor 48109, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Mar 1) 271 (9) 4573-6. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Genetic analyses of Caenorhabditis elegans has identified three genes that function in the regulation of nematode cell death. Mammalian homologs of two of these genes, ced-9 and ced-3, have been identified and comprise proteins belonging to the Bcl-2 and **ICE** families, respectively. To date, it is unclear where the negative regulators, ced-9 and bcl-2, function relative to the death effectors, ced-3 and the mammalian ced-3 homologs, respectively. Here, the molecular order of the cell death pathway is defined. Our results establish that Bcl-2 and Bcl-xL function upstream of two members of the **ICE**/CED-3 family of cysteine proteases, Yama (CPP32/apopain) and **ICE**-LAP3 (Mch3).

L16 ANSWER 8 OF 10 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN

96:879248 The Genuine Article (R) Number: VV145. CD95 (APO-1/Fas) induces activation of SAP kinases downstream of **ICE**-like proteases.

Cahill M A; Peter M E; Kischkel F C; Chinnaiyan A M; **Dixit V M**; Krammer P H; Nordheim A (Reprint). HANNOVER MED SCH, INST MOL BIOL, D-30623 HANNOVER, GERMANY (Reprint); HANNOVER MED SCH, INST MOL BIOL, D-30623 HANNOVER, GERMANY; GERMAN CANC RES CTR, DIV IMMUNOGENET, TUMOR IMMUNOL PROGRAM, D-69120 HEIDELBERG, GERMANY; UNIV MICHIGAN, SCH MED, DEPT PATHOL, ANN ARBOR, MI 48109. ONCOGENE (21 NOV 1996) Vol. 13, No. 10, pp. 2087-2096. Publisher: STOCKTON PRESS. HOUNDMILLS, BASINGSTOKE, HAMPSHIRE, ENGLAND RG21 6XS. ISSN: 0950-9232. Pub. country: GERMANY; USA. Language: English.

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Triggering of CD95 (APO-1/Pas) on different T- and B-cell lines resulted in the induction of a number of kinases (35 kDa, 38 kDa, 46 kDa and 54 kDa) that phosphorylate c-Jun and to a lesser extent Histone H1. Activation of these kinases was independent of protein biosynthesis and preceded apoptotic DNA degradation. The kinase activation pattern was specific for CD95 triggering since a variety of physical or chemical inducers of T- and B-cell apoptosis activated different kinases. The kinase activities at 46 and 54 kDa contained members of the stress-activated family of protein kinases (JNK/SAPK). Activation of the CD95-specific set of kinases was prevented by treating cells with the **ICE**-inhibiting peptide N-benzyloxycarbonyl-Val-Ala-Asp-fluoromethyl-ketone (zVAD-fmk) or by overexpression of the cow pox virus serpin CrmA. However, despite inhibition of **ICE**-like proteases the death signal was readily initiated at the cell membrane since a CD95 death-inducing signaling complex (DISC) was formed. Thus, our results demonstrate that **ICE**-like proteases in the CD95 pathway function downstream of the DISC but upstream of SAP kinases.

L16 ANSWER 9 OF 10 MEDLINE on STN DUPLICATE 2

95370174 Document Number: 95370174. PubMed ID: 7543896. CrmA-inhibitable cleavage of the 70-kDa protein component of the U1 small nuclear ribonucleoprotein during Fas- and tumor necrosis factor-induced apoptosis. Tewari M; Beidler D R; **Dixit V M**. (Department of Pathology, University of Michigan Medical School, Ann Arbor 48109, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Aug 11) 270 (32) 18738-41. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Fas and the type I tumor necrosis factor receptor (TNF-R) are two cell surface receptors that, when stimulated with ligand or cross-linking **antibody**, trigger apoptotic cell death by a mechanism that has yet to be elucidated. The CrmA protein is a serpin family protease inhibitor than can inhibit interleukin-1 beta converting enzyme (**ICE**) and **ICE**-like proteases. We showed previously that expression of CrmA potently blocks apoptosis induced by activation of either Fas or TNF-R, implicating protease involvement in these death pathways (Tewari, M., and Dixit, V.M. (1995) J. Biol. Chem. 270, 3255-3260). Here we report that the 70-kDa component of the U1 small ribonucleoprotein (U1-70 kDa) is a proteolytic substrate rapidly cleaved during both Fas- and TNF-R-induced apoptosis. This cleavage was inhibited by expression of CrmA, but not by expression of an inactive point mutant of CrmA, confirming the involvement of an **ICE**-like protease. These data for the first time identify U1-70 kDa as a death substrate cleaved during Fas- and TNF-R-induced apoptosis and emphasize the importance of protease activation in the cell death pathway.

L16 ANSWER 10 OF 10 MEDLINE on STN DUPLICATE 3

95155418 Document Number: 95155418. PubMed ID: 7531702. Fas- and tumor necrosis factor-induced apoptosis is inhibited by the poxvirus crmA gene product. Tewari M; **Dixit V M**. (Department of Pathology, University of Michigan Medical School, Ann Arbor 48109.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Feb 17) 270 (7) 3255-60. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB crmA is a cowpox virus gene that encodes a protease inhibitor of the serpin family. The only reported target for the CrmA protein is the cysteine protease interleukin-1 beta converting enzyme (**ICE**). **ICE**, by virtue of its homology to the Caenorhabditis elegans cell

death protein Ced-3, has been suggested to play a fundamentally important role in mammalian apoptosis. We hypothesized that a function of crmA may be to inhibit cell death, since a major mechanism of viral clearance is the immune system-mediated induction of apoptosis of infected cells. The tumor necrosis factor receptor and the Fas antigen are two cytokine receptors which, by engaging and activating the death pathway, can eliminate virus-infected cells. Remarkably, crmA was found to be an exceptionally potent inhibitor of apoptosis induced by both these receptors, capable of blocking the cell death program even at pharmacological doses of the death stimulus. Therefore, an important new function for crmA is the inhibition of cytokine-induced apoptosis. Further, the data suggest that a protease, either **ICE** or a related crmA-inhibitable protein, is a component of the Fas- and tumor necrosis factor-induced cell death pathways.

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L3   ANSWER 1 OF 5  BIOSIS  COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
2001:563673  Document No.: PREV200100563673. Interleukin-1 beta converting
     enzyme like apoptotic protease-6. Dixit, Vishva M. [Inventor]; He, Wei-Wu
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[Inventor]; Kikly, Kristine K. [Inventor]; **Ruben, Steven M.** [Inventor]. ASSIGNEE: Human Genome Sciences, Inc.; SmithKline Beecham Corporation; University of Michigan. Patent Info.: US 6294169 September 25, 2001. Official Gazette of the United States Patent and Trademark Office Patents, (Sep. 25, 2001) Vol. 1250, No. 4. e-file.
CODEN: OGUPE7. ISSN: 0098-1133. Language: English.

AB Human **ICE** LAP-6 polypeptides and DNA (RNA) encoding such **ICE** LAP-6 and a procedure for producing such polypeptides by recombinant techniques is disclosed. Also disclosed are methods for utilizing such **ICE** LAP-6 for the treatment of a susceptibility to viral infection, tumorogenesis and to diseases and defects in the control embryogenesis and tissue homeostasis, and the nucleic acid sequences described above may be employed in an assay for ascertaining such susceptibility. Antagonists against such **ICE** LAP-6 and their use as a therapeutic to treat Alzheimer's disease, Parkinson's disease, rheumatoid arthritis, septic shock, sepsis, stroke, chronic inflammation, acute inflammation, CNS inflammation, osteoporosis, ischemia reperfusion injury, cell death associated with cardiovascular disease, polycystic kidney disease, apoptosis of endothelial cells in cardiovascular disease, degenerative liver disease, MS, ALS, cererbellar degeneration, ischemic injury, myocardial infarction, AIDS, myelodysplastic syndromes, aplastic anemia, male pattern baldness, and head injury damage are also disclosed. Also disclosed are diagnostic assays for detecting diseases related to mutations in the nucleic acid sequences and altered concentrations of the polypeptides. Also disclosed are diagnostic assays for detecting mutations in the polynucleotides encoding the interleukin-1 beta converting enzyme apoptosis proteases and for detecting altered levels of the polypeptide in a host.

L3 ANSWER 2 OF 5 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
2000:305066 Document No.: PREV200000305066. Interleukin-1 beta converting enzyme like apoptotic protease-6. Dixit, Vishva M. [Inventor, Reprint author]; He, Wei-Wu [Inventor]; Kikly, Kristine K. [Inventor]; **Ruben, Steven M.** [Inventor]. AnnArbor, MI, USA. ASSIGNEE: Smithkline Beecham Corporation. Patent Info.: US 6010878 January 04, 2000. Official Gazette of the United States Patent and Trademark Office Patents, (Jan. 4, 2000) Vol. 1230, No. 1. e-file.
CODEN: OGUPE7. ISSN: 0098-1133. Language: English.

AB Human **ICE** LAP-6 polypeptides and DNA (RNA) encoding such **ICE** LAP-6 and a procedure for producing such polypeptides by recombinant techniques is disclosed. Also disclosed are methods for utilizing such **ICE** LAP-6 for the treatment of a susceptibility to viral infection, tumorogenesis and to diseases and defects in the control embryogenesis and tissue homeostasis, and the nucleic acid sequences described above may be employed in an assay for ascertaining such susceptibility. Antagonists against such **ICE** LAP-6 and their use as a therapeutic to treat Alzheimer's disease, Parkinson's disease, rheumatoid arthritis, septic shock, sepsis, stroke, chronic inflammation, acute inflammation, CNS inflammation, osteoporosis, ischemia reperfusion injury, cell death associated with cardiovascular disease, polycystic kidney disease, apoptosis of endothelial cells in cardiovascular disease, degenerative liver disease, MS, ALS, cererbellar degeneration, ischemic injury, myocardial infarction, AIDS, myelodysplastic syndromes, aplastic anemia, male pattern baldness, and head injury damage are also disclosed. Also disclosed are diagnostic assays for detecting diseases related to mutations in the nucleic acid sequences and altered concentrations of the polypeptides. Also disclosed are diagnostic assays for detecting mutations in the polynucleotides encoding the interleukin-1 beta converting enzyme apoptosis proteases and for detecting altered levels of the polypeptide in a host.

L3 ANSWER 3 OF 5 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
2000:301605 Document No.: PREV200000301605. Interleukin-1 beta converting enzyme like apoptotic protease-7. Dixit, Vishva M. [Inventor, Reprint author]; Kikly, Kristine K. [Inventor]; **Ruben, Stev n M.**

[Inventor]; Ni, Jian [Inventor]; Rosen, Craig A. [Inventor]. Ann Arbor, MI, USA. ASSIGNEE: Smithkline Beecham Corporation, Philadelphia, PA, USA; The Regents of the University of Michigan; Human Genome Sciences, Inc.. Patent Info.: US 6008042 December 28, 1999. Official Gazette of the United States Patent and Trademark Office Patents, (Dec. 28, 1999) Vol. 1229, No. 4. e-file.
CODEN: OGUPE7. ISSN: 0098-1133. Language: English.

AB Human **ICE** LAP-7 polypeptides and DNA (RNA) encoding such **ICE** LAP-7 and a procedure for producing such polypeptides by recombinant techniques is disclosed. Also disclosed are methods for utilizing such **ICE** LAP-7 for the treatment of a susceptibility to viral infection, tumorogenesis and to diseases and defects in the control embryogenesis and tissue homeostasis, and the nucleic acid sequences described above may be employed in an assay for ascertaining such susceptibility. Antagonists against such **ICE** LAP-7 and their use as a therapeutic to treat Alzheimer's disease, Parkinson's disease, rheumatoid arthritis, septic shock, sepsis, stroke, chronic inflammation, acute inflammation, CNS inflammation, osteoporosis, ischemia reperfusion injury, cell death associated with cardiovascular disease, polycystic kidney disease, apoptosis of endothelial cells in cardiovascular disease, degenerative liver disease, MS, ALS, cererbellar degeneration, ischemic injury, myocardial infarction, AIDS, myelodysplastic syndromes, aplastic anemia, male pattern baldness, and head injury damage are also disclosed. Also disclosed are diagnostic assays for detecting diseases related to mutations in the nucleic acid sequences and altered concentrations of the polypeptides. Also disclosed are diagnostic assays for detecting mutations in the polynucleotides encoding the interleukin-1 beta converting enzyme apoptosis proteases and for detecting altered levels of the polypeptide in a host.

L3 ANSWER 4 OF 5 CAPLUS COPYRIGHT 2003 ACS on STN

1997:761953 Document No. 128:31833 Cloning of human interleukin-1.beta. converting enzyme-like apoptotic protease-6 and its diagnostic and therapeutic applications. Dixit, Vishva M.; He, Wei-wu; **Ruben, Steven M.**; Kikly, Kristine K. (Smithkline Beecham Corp., USA; Human Genome Sciences, Inc.; University of Michigan). Eur. Pat. Appl. EP 808904 A2 19971126, 44 pp. DESIGNATED STATES: R: BE, CH, DE, DK, FR, GB, IT, LI, NL. (English). CODEN: EPXXDW. APPLICATION: EP 1997-303397 19970519. PRIORITY: US 1996-17949 19960520; US 1996-20344 19960523; US 1996-18961 19960605.

AB Members of the **ICE**/Ced-3 gene family are likely effector components of the cell death machinery. A novel member of this family designated **ICE**-LAP-6 is provided. By phylogenetic anal., **ICE**-LAP6 is classified into the Ced-3 subfamily which includes Ced-3, Yama/CPP32/apopain, Mch2, and **ICE**-LAP3/Mch3/CMH-1. Interestingly, **ICE**-LAP6 contains an active site QACGG pentapeptide, rather than the QACRG pentapeptide shared by other family members. Overexpression of **ICE**-LAP6 induces apoptosis in MCF7 breast carcinoma cells. More importantly, **ICE**-LAP6 is proteolytically processed into an active cysteine protease by granzyme B, an important component of cytotoxic T cell-mediated apoptosis. Once activated, **ICE**-LAP6 is able to cleave the death substrate poly(ADP-ribose) polymerase into signature apoptotic fragments. Also disclosed are methods for utilizing such **ICE** LAP-6 for the treatment of a susceptibility to viral infection, tumorigenesis, and to diseases and defects in the control embryogenesis and tissue homeostasis, and the nucleic acid sequences described may be employed in an assay for ascertaining such susceptibility. Agonists and antagonists of **ICE** LAP-6 may also be used to treat various disease states.

L3 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2003 ACS on STN

1997:761853 Document No. 128:31110 Cloning and cDNA sequence of human interleukin-1.beta. converting enzyme-like apoptotic protease 7. Dixit, Vishva M.; Kikly, Kristine K.; Ni, Jian; Rosen, Craig A.; **Ruben, Steven M.** (Smithkline Beecham Corp., USA; Human Genome Sciences,

Inc.; University of Michigan). Eur. Pat. Appl. EP 807686 A2 19971119, 48 pp. DESIGNATED STATES: R: BE, CH, DE, DK, FR, GB, IT, LI, NL. (English). CODEN: EPXXDW. APPLICATION: EP 1997-303203 19970512. PRIORITY: US 1996-17914 19960516; US 1996-17454 19960517; US 1996-19365 19960605.

AB Human interleukin-1.beta. converting enzyme apoptosis protease-7 (**ICE** LAP-7, or FLICE) polypeptides and DNA (RNA) encoding such **ICE** LAP-7 and a procedure for producing such polypeptides by recombinant techniques is disclosed. Also disclosed are methods for utilizing such **ICE** LAP-7 for the treatment of a susceptibility to viral infection, tumorigenesis, and to diseases and defects in the control embryogenesis and tissue homeostasis, and the nucleic acid sequences described above may be employed in an assay for ascertaining such susceptibility. Antagonists against such **ICE** LAP-7 and their use as a therapeutic to treat Alzheimer's disease, Parkinson's disease, rheumatoid arthritis, septic shock, sepsis, stroke, chronic inflammation, acute inflammation, CNS inflammation, osteoporosis, ischemia reperfusion injury, cell death assocd. with cardiovascular disease, polycystic kidney disease, apoptosis of endothelial cells in cardiovascular disease, degenerative liver disease, MS, ALS, cerebellar degeneration, ischemic injury, myocardial infarction, AIDS, myelodysplastic syndromes, aplastic anemia, male pattern baldness, and head injury damage are also disclosed. Also disclosed are diagnostic assays for detecting diseases related to mutations in the nucleic acid sequences and altered concns. of the polypeptides. Also disclosed are diagnostic assays for detecting mutations in the polynucleotides encoding the interleukin-1.beta. converting enzyme apoptosis proteases and for detecting altered levels of the polypeptide in a host.

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L4          527 L1 AND ANTIBOD?

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L5 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2003 ACS on STN

1997:761953 Document No. 128:31833 Cloning of human interleukin-1.beta. converting enzyme-like apoptotic protease-6 and its diagnostic and therapeutic applications. Dixit, Vishva M.; He, Wei-wu; **Ruben, Steven M.**; Kikly, Kristine K. (Smithkline Beecham Corp., USA; Human Genome Sciences, Inc.; University of Michigan). Eur. Pat. Appl. EP 808904 A2 19971126, 44 pp. DESIGNATED STATES: R: BE, CH, DE, DK, FR, GB, IT, LI, NL. (English). CODEN: EPXXDW. APPLICATION: EP 1997-303397 19970519. PRIORITY: US 1996-17949 19960520; US 1996-20344 19960523; US 1996-18961 19960605.

AB Members of the ICE/Ced-3 gene family are likely effector components of the cell death machinery. A novel member of this family designated **ICE-LAP-6** is provided. By phylogenetic anal., ICE-LAP6 is classified into the Ced-3 subfamily which includes Ced-3, Yama/CPP32/apopain, Mch2, and ICE-LAP3/Mch3/CMH-1. Interestingly, ICE-LAP6 contains an active site QACGG pentapeptide, rather than the QACRG pentapeptide shared by other family members. Overexpression of ICE-LAP6 induces apoptosis in MCF7 breast carcinoma cells. More importantly, ICE-LAP6 is proteolytically processed into an active cysteine protease by granzyme B, an important component of cytotoxic T cell-mediated apoptosis. Once activated, ICE-LAP6 is able to cleave the death substrate poly(ADP-ribose) polymerase into signature apoptotic fragments. Also disclosed are methods for utilizing such **ICE LAP-6** for the treatment of a susceptibility to viral infection, tumorigenesis, and to diseases and defects in the control embryogenesis and tissue homeostasis, and the nucleic acid sequences described may be employed in an assay for ascertaining such susceptibility. Agonists and

antagonists of **ICE LAP-6** may also be used to
treat various disease states.

=> s antibody and CPP32
L6 1030 ANTIBODY AND CPP32

=> s l6 and apopain
L7 85 L6 AND APOPAIN

=> s l7 and Mch2
L8 1 L7 AND MCH2

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L8 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2003 ACS on STN
1997:761953 Document No. 128:31833 Cloning of human interleukin-1.beta. converting enzyme-like apoptotic protease-6 and its diagnostic and therapeutic applications. Dixit, Vishva M.; He, Wei-wu; Ruben, Steven M.; Kikly, Kristine K. (Smithkline Beecham Corp., USA; Human Genome Sciences, Inc.; University of Michigan). Eur. Pat. Appl. EP 808904 A2 19971126, 44 pp. DESIGNATED STATES: R: BE, CH, DE, DK, FR, GB, IT, LI, NL. (English). CODEN: EPXXDW. APPLICATION: EP 1997-303397 19970519. PRIORITY: US 1996-17949 19960520; US 1996-20344 19960523; US 1996-18961 19960605.
AB Members of the ICE/Ced-3 gene family are likely effector components of the cell death machinery. A novel member of this family designated ICE-LAP-6 is provided. By phylogenetic anal., ICE-LAP6 is classified into the Ced-3 subfamily which includes Ced-3, Yama/**CPP32**/**apopain**, **Mch2**, and ICE-LAP3/Mch3/CMH-1. Interestingly, ICE-LAP6 contains an active site QACGG pentapeptide, rather than the QACRG pentapeptide shared by other family members. Overexpression of ICE-LAP6 induces apoptosis in MCF7 breast carcinoma cells. More importantly, ICE-LAP6 is proteolytically processed into an active cysteine protease by granzyme B, an important component of cytotoxic T cell-mediated apoptosis. Once activated, ICE-LAP6 is able to cleave the death substrate poly(ADP-ribose) polymerase into signature apoptotic fragments. Also disclosed are methods for utilizing such ICE LAP-6 for the treatment of a susceptibility to viral infection, tumorigenesis, and to diseases and defects in the control embryogenesis and tissue homeostasis, and the nucleic acid sequences described may be employed in an assay for ascertaining such susceptibility. Agonists and antagonists of ICE LAP-6 may also be used to treat various disease states.

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L9 ANSWER 1 OF 35 CAPLUS COPYRIGHT 2003 ACS on STN
2000:594886 Document No. 134:84283 Reduced expression of ICE/caspase1 and **CPP32**/caspase3 in human hepatocellular carcinoma. Fujikawa, Katsuhiko; Shiraki, Katsuya; Sugimoto, Kazushi; Ito, Takeshi; Yamanaka, Takenari; Takase, Koujiro; Nakano, Takeshi (First Department of Internal Medicine, Mie University School of Medicine, Mie, 514-8507, Japan). Anticancer Research, 20(3B), 1927-1932 (English) 2000. CODEN: ANTRD4. ISSN: 0250-7005. Publisher: International Institute of Anticancer Research.
AB The interleukin-1-converting enzyme (ICE)/caspase1 and the **CPP32** /caspase3, cysteine protease, play an important role in the maintenance of homeostasis by inducing apoptosis. Since human hepatocellular carcinomas (HCCs) demonstrate strong resistance to apoptosis, the authors investigated the expression of ICE and **CPP32** in human HCCs.

Reverse transcription PCR anal. revealed that one out of five HCC tissues showed no band of ICE mRNA and two out of five HCC tissues showed no band of **CPP32** mRNA. An immuno-histochem. study of 20 cases of HCC tissues and non-tumor parts revealed that immunoreactivity of ICE and **CPP32** was preferentially obsd. in the cytoplasm, appearing as a diffuse and homogeneous pattern. Some nuclei also stained with anti-ICE **antibody** or anti-**CPP32** **antibody** and demonstrated apoptotic features. Overall, the expression of ICE and **CPP32** were significantly down-regulated in the HCCs compared to non-tumor cells. In situ nick end labeling method (TUNEL) labeling index significantly decreased according to the decreasing staining intensity of **CPP32**. However, there was no tendency for the TUNEL labeling index to decrease with decreasing ICE staining intensity. The authors' results suggested that the expression of ICE and **CPP32** were down-regulated and that esp. reduced expression of **CPP32** may contribute to resistance against apoptosis in human HCCs.

L9 ANSWER 2 OF 35 CAPLUS COPYRIGHT 2003 ACS on STN
2000:14384 Document No. 132:288724 Pentoxifylline inhibits anti-Fas **antibody**-induced hepatitis by affecting downstream of **CPP32**-like activity in mice. Okamoto, Toshihiro (Research Laboratories, Nippon Chemiphar Co., Ltd., Saitama, 341-0005, Japan). International Journal of Molecular Medicine, 4(6), 601-603 (English) 1999. CODEN: IJMMFG. ISSN: 1107-3756. Publisher: International Journal of Molecular Medicine.

AB The effect of pentoxifylline on anti-Fas **antibody**-induced hepatitis was studied. The administration of anti-Fas **antibodies** (250 .mu.g/kg, i.v.) to mice elevated plasma alanine aminotransferase (ALT) activity at 3 h. This anti-Fas **antibody**-induced elevation of ALT was inhibited by treatment with pentoxifylline at the doses of 10 and 100 mg/kg (i.p.). Anti-Fas **antibody** administration also elevated the **CPP32**-like protease activity in the liver at 3 h. Although pentoxifylline at 100 mg/kg, i.p., inhibited the anti-Fas **antibody**-induced elevation of plasma ALT, this treatment did not significantly inhibit the anti-Fas **antibody**-induced elevation of **CPP32**-like activity. The present results clearly showed that treatment with pentoxifylline inhibited anti-Fas **antibody** -induced hepatitis, at least in part, by affecting a reaction downstream of **CPP32**-like protease activation.

L9 ANSWER 3 OF 35 CAPLUS COPYRIGHT 2003 ACS on STN
1999:273082 Document No. 131:67829 The protective effect of cyclosporine A on anti-fas **antibody**-induced hepatitis in mice. Okamoto, Toshihiro; Hitomi, Yuji; Hara, Atsuko (Research Laboratories, Nippon Chemiphar Co., Ltd., Saitama, 341-0005, Japan). Japanese Journal of Pharmacology, 79(4), 485-488 (English) 1999. CODEN: JJPAAZ. ISSN: 0021-5198. Publisher: Japanese Pharmacological Society.

AB The effect of cyclosporine A (CsA) on anti-Fas **antibody**-induced hepatitis was studied. The administration of anti-Fas **antibody** (250 .mu.g/kg) to mice elevated plasma alanine aminotransferase (ALT) activity at 3 h. This anti-Fas **antibody**-induced elevation of ALT was inhibited by treatment with CsA (10, 30 and 100 mg/kg) in a dose-dependent manner. Anti-Fas **antibody** administration elevated **CPP32**-like protease activity at 3 h in mouse liver, and this elevation of **CPP32**-like activity was inhibited by treatment with CsA. The present results show that CsA treatment inhibits the anti-Fas **antibody**-induced apoptotic process of hepatitis, at least in part, by affecting a reaction upstream of **CPP32**-like protease activation.

L9 ANSWER 4 OF 35 MEDLINE on STN DUPLICATE 1
1999313557 Document Number: 99313557. PubMed ID: 10386985. Activation of caspase-3 in developmental models of programmed cell death in neurons of the substantia nigra. Jeon B S; Kholodilov N G; Oo T F; Kim S Y; Tomaselli K J; Srinivasan A; Stefanis L; Burke R E. (Department of Neurology,

Columbia University College of Physicians and Surgeons, New York, New York 10032, USA.) JOURNAL OF NEUROCHEMISTRY, (1999 Jul) 73 (1) 322-33. Journal code: 2985190R. ISSN: 0022-3042. Pub. country: United States. Language: English.

AB Programmed cell death has been proposed to play a role in the death of neurons in acute and chronic degenerative neurologic disease. There is now evidence that the caspases, a family of cysteine proteases, mediate programmed cell death in various cells. In neurons, caspase-3 (**CPP32**/Yama/**apopain**), in particular, has been proposed to play a role. We examined the expression of caspase-3 in three models of programmed cell death affecting neurons of the substantia nigra in the rat: natural developmental neuron death and induced developmental death following either striatal target injury with quinolinic acid or dopamine terminal lesion with intrastriatal injection of 6-hydroxydopamine. Using an **antibody** to the large (p17) subunit of activated caspase-3, we have found that activated enzyme is expressed in apoptotic profiles in all models. Increased p17 immunostaining correlated with increased enzyme activity. The subcellular distribution of activated caspase-3 differed among the models: In natural cell death and the target injury model, it was strictly nuclear, whereas in the toxin model, it was also cytoplasmic. We conclude that p17 immunostaining is a useful marker for programmed cell death in neurons of the substantia nigra.

L9 ANSWER 5 OF 35 CAPLUS COPYRIGHT 2003 ACS on STN

1999:248679 Document No. 131:56930 Activation of caspases in p53-induced transactivation-independent apoptosis. Gao, Chongfeng; Tsuchida, Nobuo (Department of Molecular Cellular Oncology, Tokyo Medical and Dental University, Tokyo, 113-8549, Japan). Japanese Journal of Cancer Research, 90(2), 180-187 (English) 1999. CODEN: JJCREP. ISSN: 0910-5050. Publisher: Japanese Cancer Association.

AB Though p53-induced apoptosis plays an important role in tumor suppression, the mechanism(s) by which p53 induces apoptosis is still unclear. To elucidate the p53-induced apoptotic pathway, the authors examd. the role of p53 transactivation activity and caspase in J138V5C cells carrying a human temp.-sensitive (ts) p53 mutant (138Ala.fwdarw.Val). The results showed that p53-induced apoptosis was not blocked by cycloheximide, which effectively prevented the expression of p53 target genes, indicating that transactivation was not essential for p53-induced apoptosis in this system. Western blot anal. showed that PARP, **CPP32** and ICH-1 precursors were cleaved during apoptosis. The **CPP32** -preferential tetrapeptide inhibitor Ac-DEVD-CHO blocked the cleavage of ICH-1 and PARP precursors, suggesting that **CPP32** or some other DEVD-sensitive caspase(s) is the upstream activator of ICH-1. The authors also examd. the role of the Fas pathway by using Fas and Fas ligand-neutralizing **antibodies**. Both **antibodies** failed to block p53-induced apoptosis, suggesting that the Fas pathway was not essential for p53-induced apoptosis in this system. Taken together, the authors' results indicate that p53-induced, transactivation-independent apoptosis in Jurkat cells involves sequential activation of **CPP32** or some other DEVD-sensitive caspase(s) and ICH-1, via a Fas-independent pathway.

L9 ANSWER 6 OF 35 MEDLINE on STN

1999047701 Document Number: 99047701. PubMed ID: 9830064. TRAIL/Apo2L activates c-Jun NH2-terminal kinase (JNK) via caspase-dependent and caspase-independent pathways. Muhlenbeck F; Haas E; Schwenzer R; Schubert G; Grell M; Smith C; Scheurich P; Wajant H. (Institute of Cell Biology and Immunology, University of Stuttgart, Allmandring 31, 70569 Stuttgart, Germany.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Dec 4) 273 (49) 33091-8. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB In this study we show that TRAIL (tumor necrosis factor-related apoptosis-inducing ligand), also called Apo2L, activates the c-Jun N-terminal kinase (JNK). Interestingly, TRAIL-induced JNK activation occurs in a cell type-specific manner. In HeLa cells, TRAIL-induced JNK

activation can be completely blocked with the cysteine protease inhibitor zVAD-fmk, whereas the same inhibitor has no, or even a stimulatory, effect on JNK activation in Kym-1 cells. Hence, TRAIL can engage at least two independent pathways leading to JNK activation, one that is cysteine protease-dependent and one that is cysteine protease-independent. To investigate whether the cysteine protease-dependent signaling of TRAIL leading to JNK activation is related to the apoptotic pathway engaged by this ligand, we investigated HeLa cells stably overexpressing a dominant negative mutant of FADD (Fas-associating protein with death domain) (GFP(green fluorescent protein)DeltaFADD). In these cells, TRAIL-induced cell death and activation of the apoptosis executioner caspase-8 (FLICE/MACH) and caspase-3 (YAMA, CPP-32, **Apopain**), that belong to caspase subfamily of cysteine proteases, were abrogated, whereas JNK activation remained unaffected and was still sensitive toward z-VAD-fmk. Similar data were found in HeLa cells overexpressing Apo1/Fas and GFPDeltaFADD upon stimulation with agonistic **antibodies**. These data suggest that cross-linking of the TRAIL receptors and Apo1/Fas, respectively, engages a FADD-dependent pathway leading to the activation of apoptotic caspases and, in parallel, a FADD-independent pathway leading to the stimulation of one or more cysteine proteases capable to activate JNK but not sufficient for the induction of cell death.

L9 ANSWER 7 OF 35 MEDLINE on STN DUPLICATE 2

1999222074 Document Number: 99222074. PubMed ID: 10203687. Activation of the CD95 (APO-1/Fas) pathway in drug- and gamma-irradiation-induced apoptosis of brain tumor cells. Fulda S; Scaffidi C; Pietsch T; Krammer P H; Peter M E; Debatin K M. (University Children's Hospital, Prittwitzstr. 43, D-89075 Ulm, Germany.) CELL DEATH AND DIFFERENTIATION, (1998 Oct) 5 (10) 884-93. Journal code: 9437445. ISSN: 1350-9047. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Chemotherapeutic agents and gamma-irradiation used in the treatment of brain tumors, the most common solid tumors of childhood, have been shown to act primarily by inducing apoptosis. Here, we report that activation of the CD95 pathway was involved in drug- and gamma-irradiation-induced apoptosis of medulloblastoma and glioblastoma cells. Upon treatment CD95 ligand (CD95-L) was induced that stimulated the CD95 pathway by crosslinking CD95 via an autocrine/paracrine loop. Blocking CD95-L/receptor interaction using F(ab')2 anti-CD95 **antibody** fragments strongly reduced apoptosis. Apoptosis depended on activation of caspases (interleukin 1beta-converting enzyme/Ced-3 like proteases) as it was almost completely abrograted by the broad range caspase inhibitor benzyloxycarbonyl-Val-Ala-Asp-fluoromethyl ketone. Apoptosis was mediated by cleavage of the receptor proximal caspase FLICE/MACH (caspase-8) and the downstream caspase **CPP32** (caspase-3, **Apopain**) resulting in cleavage of the prototype caspase substrate PARP. Moreover, CD95 was upregulated in wild-type p53 cells thereby increasing responsiveness towards CD95 triggering. Since activation of the CD95 system upon treatment was also found in primary medulloblastoma cells ex vivo, these findings may have implications to define chemosensitivity and to develop novel therapeutic strategies in the management of malignant brain tumors.

L9 ANSWER 8 OF 35 MEDLINE on STN

1998444383 Document Number: 98444383. PubMed ID: 9767416. Peroxynitrite-induced thymocyte apoptosis: the role of caspases and poly (ADP-ribose) synthetase (PARS) activation. Virag L; Scott G S; Cuzzocrea S; Marmer D; Salzman A L; Szabo C. (Division of Critical Care, Children's Hospital Medical Center, Cincinnati, OH 45229, USA.) IMMUNOLOGY, (1998 Jul) 94 (3) 345-55. Journal code: 0374672. ISSN: 0019-2805. Pub. country: ENGLAND: United Kingdom. Language: English.

AB The mechanisms by which immature thymocyte apoptosis is induced during negative selection are poorly defined. Reports demonstrated that cross-linking of T-cell receptor leads to stromal cell activation, expression of inducible nitric oxide synthase (iNOS) and, subsequently, to thymocyte apoptosis. Therefore we examined, whether NO directly or

indirectly, through peroxynitrite formation, causes thymocyte apoptosis. Immuno-histochemical detection of nitrotyrosine revealed in vivo peroxynitrite formation in the thymi of naive mice. Nitrotyrosine, the footprint of peroxynitrite, was predominantly found in the corticomedullary junction and the medulla of naive mice. In the thymi of mice deficient in the inducible isoform of nitric oxide synthase, considerably less nitrotyrosine was found. Exposure of thymocytes in vitro to low concentrations (10 microM) of peroxynitrite led to apoptosis, whereas higher concentrations (50 microM) resulted in intense cell death with the characteristics of necrosis. We also investigated the effect of poly (ADP-ribose) synthetase (PARS) inhibition on thymocyte apoptosis. Using the PARS inhibitor 3-aminobenzamide (3-AB), or thymocytes from PARS-deficient animals, we established that PARS determines the fate of thymocyte death. Suppression of cellular ATP levels, and the cellular necrosis in response to peroxynitrite were prevented by PARS inhibition. Therefore, in the absence of PARS, cells are diverted towards the pathway of apoptotic cell death. Similar results were obtained with H2O2 treatment, while apoptosis induced by non-oxidative stimuli such as dexamethasone or anti-FAS **antibody** was unaffected by PARS inhibition. In conclusion, we propose that peroxynitrite-induced apoptosis may play a role in the process of thymocyte negative selection. Furthermore, we propose that the physiological role of PARS cleavage by **apopain** during apoptosis may serve as an energy-conserving step, enabling the cell to complete the process of apoptosis.

L9 ANSWER 9 OF 35 MEDLINE on STN

1999218585 Document Number: 99218585. PubMed ID: 10200474. Wortmannin enhances activation of **CPP32** (Caspase-3) induced by TNF or anti-Fas. Fujita E; Kouroku Y; Miho Y; Tsukahara T; Ishiura S; Momoi T. (Division of Development and Differentiation, National Institute of Neuroscience, NCNP, Kodaira, Tokyo 187, Japan.) CELL DEATH AND DIFFERENTIATION, (1998 Apr) 5 (4) 289-97. Journal code: 9437445. ISSN: 1350-9047. Pub. country: ENGLAND: United Kingdom. Language: English.

AB **CPP32/apopain** (Caspase-3), a protease of the Ced-3/ICE family, is a central mediator in the apoptosis induced by TNF or anti-Fas. In this study we demonstrate that wortmannin, an inhibitor of PI-3K, enhances the activation of **CPP32** (Caspase-3) and DNA fragmentation in TNF-treated U937 cells and anti-Fas-treated Jurkat cells. Caspase-3-like activity, Ac-DEVD-MCA cleavage activity, is enhanced by wortmannin in the range of the concentration (1 - 100 nM) specifically inhibiting PI-3K. LY294002, another PI-3K inhibitor, also enhances Caspase-3-like activity, but inhibitors for myosin light chain kinase and calmodulin dependent kinase do not have any effect on the Caspase-3-like activity. Wortmannin (1 - 100 nM) enhances the processing of Caspase-3 (32K) into active form (17K) in TNF- or anti-Fas-treated cells, but not in untreated cells. These observations suggest that inhibition of PI-3K induces the activation of processing enzyme of Caspase-3 or increases the susceptibility of Caspase-3 to the processing enzyme. PI-3K seems to protect the cells from apoptosis by suppressing the activation of Caspase-3.

L9 ANSWER 10 OF 35 MEDLINE on STN DUPLICATE 3

1998330892 Document Number: 98330892. PubMed ID: 9666463. Alleviation of apoptosis by serum in Chinese hamster ovary cells ectopically expressing human Fas antigen. Lee Y S; Nakajima H; Chang Y C; Park K I; Mitsui Y; Magae J; Saida K. (National Institute of Bioscience and Human-Technology, Tsukuba, Japan.) MOLECULES AND CELLS, (1998 Jun 30) 8 (3) 272-9. Journal code: 9610936. ISSN: 1016-8478. Pub. country: KOREA. Language: English.

AB Fas-mediated apoptosis is an important regulatory mechanism for the development of T-cells and prevention of oncogenesis. Here, we establish Chinese hamster ovary (CHO) cell lines which stably express Fas antigen, and analyzed apoptosis induced by anti-Fas IgM. While Fas-transfected hamster cells did not undergo apoptosis when stimulated with anti-Fas **antibody** in the presence of medium containing 10% serum, in reduced serum concentrations, anti-Fas **antibody** caused these

cells to round up and detach from the culture dish. Analysis of the DNA content by a flow cytometry demonstrated a significant increase of cells with sub-G1 amount of DNA upon Fas stimulation in the low serum concentrations. The increase in the number of apoptosis cells was inhibited by an **apopain** (**CPP32**, caspase 3) inhibitor or insulin-like growth factor-I. In contrast, apoptosis in a Fas-transfected mouse T-cell line occurred in the presence of 10% serum. these results suggest that factors including insulin-like growth factor-I in fetal bovine serum protect CHO cells from **apopain**-dependent apoptosis mediated by Fas-antigen stimulation.

L9 ANSWER 11 OF 35 CAPLUS COPYRIGHT 2003 ACS on STN
1998:29898 Document No. 128:126893 Genetically recessive mutant of human monocytic leukemia U937 resistant to tumor necrosis factor-.alpha.-induced apoptosis. Dong, Jian; Naito, Mikihiko; Mashima, Tetsuo; Jang, Won Hee; Tsuruo, Akashi (Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo, Japan). Journal of Cellular Physiology, 174(2), 179-185 (English) 1998. CODEN: JCLLAX. ISSN: 0021-9541. Publisher: Wiley-Liss, Inc..
AB Tumor necrosis factor-.alpha. (TNF-.alpha.) is a cytokine that induces apoptosis in various cell systems by binding to the TNF receptor (TNFR). To study TNF-.alpha.-induced apoptosis, we isolated and characterized a novel TNF-.alpha.-resistant variant, U937/TNF clone UA, from human monocytic leukemia U937 cells. The UA cells resist apoptosis induced by TNF-.alpha. and anti-Fas **antibody** but not by anticancer drugs, such as VP-16 and Ara-C. Somatic cell hybridization between U937 and UA showed that apoptosis resistance to TNF-.alpha. in UA was genetically recessive. The hybridization anal. also showed that UA and another recessive mutant clone, UC, belong to different complementation groups in TNF-.alpha.-induced apoptosis signaling. In UA cells, TNF-.alpha.-induced disruption of mitochondrial membrane potential and **CPP32** activation were abrogated. Expression of TNFR, Fas, and Bcl-2 family proteins was not changed in UA cells. These results suggest that the apoptosis resistant UA cells could have a functional defect in apoptosis signaling from the TNFR to mitochondria and interleukin-1.beta. converting enzyme (ICE) family protease activation. UA cells could be used to study signaling linkage between cell death-inducing receptor and mitochondria.

L9 ANSWER 12 OF 35 CAPLUS COPYRIGHT 2003 ACS on STN
1998:275996 Document No. 129:52599 Proteolytic cleavage of retinoblastoma protein upon DNA damage and Fas-mediated apoptosis. Diederich, L.; Fotedar, A.; Fotedar, R. (Institut Biologie Structurale J.-P. Ebel, Grenoble, Fr.). Cell Biology and Toxicology, 14(2), 133-140 (English) 1998. CODEN: CBTOE2. ISSN: 0742-2091. Publisher: Kluwer Academic Publishers.
AB Proteolytic cleavage of key cellular proteins by caspases (ICE, **CPP32**, and Ich-1/Nedd2) may be crucial to the apoptotic process. The retinoblastoma tumor suppressor gene is a neg. regulator of cell growth and the retinoblastoma protein (pRb) exhibits anti-apoptotic function. We show that pRb is cleaved during apoptosis induced by either UV irradn. or anti-Fas **antibody**. Our studies implicate **CPP32**-like activity in the proteolytic cleavage of pRb. The kinetics of proteolytic cleavage of pRb during apoptosis differ from that obsd. for other cellular proteins, suggesting that the specific cleavage of pRb during apoptosis may be an important event.

L9 ANSWER 13 OF 35 CAPLUS COPYRIGHT 2003 ACS on STN
1997:761953 Document No. 128:31833 Cloning of human interleukin-1.beta. converting enzyme-like apoptotic protease-6 and its diagnostic and therapeutic applications. Dixit, Vishva M.; He, Wei-wu; Ruben, Steven M.; Kikly, Kristine K. (Smithkline Beecham Corp., USA; Human Genome Sciences, Inc.; University of Michigan). Eur. Pat. Appl. EP 808904 A2 19971126, 44 pp. DESIGNATED STATES: R: BE, CH, DE, DK, FR, GB, IT, LI, NL. (English). CODEN: EPXXDW. APPLICATION: EP 1997-303397 19970519. PRIORITY: US 1996-17949 19960520; US 1996-20344 19960523; US 1996-18961

19960605.

AB Members of the ICE/Ced-3 gene family are likely effector components of the cell death machinery. A novel member of this family designated ICE-LAP-6 is provided. By phylogenetic anal., ICE-LAP6 is classified into the Ced-3 subfamily which includes Ced-3, Yama/**CPP32**/**apopain**, Mch2, and ICE-LAP3/Mch3/CMH-1. Interestingly, ICE-LAP6 contains an active site QACGG pentapeptide, rather than the QACRG pentapeptide shared by other family members. Overexpression of ICE-LAP6 induces apoptosis in MCF7 breast carcinoma cells. More importantly, ICE-LAP6 is proteolytically processed into an active cysteine protease by granzyme B, an important component of cytotoxic T cell-mediated apoptosis. Once activated, ICE-LAP6 is able to cleave the death substrate poly(ADP-ribose) polymerase into signature apoptotic fragments. Also disclosed are methods for utilizing such ICE LAP-6 for the treatment of a susceptibility to viral infection, tumorigenesis, and to diseases and defects in the control embryogenesis and tissue homeostasis, and the nucleic acid sequences described may be employed in an assay for ascertaining such susceptibility. Agonists and antagonists of ICE LAP-6 may also be used to treat various disease states.

L9 ANSWER 14 OF 35 CAPLUS COPYRIGHT 2003 ACS on STN

1997:510351 Document No. 127:119961 The large subunit of the DNA replication complex C (DSEB/RF-C140) cleaved and inactivated by caspase-3 (**CPP32**/YAMA) during Fas-induced apoptosis. Ubeda, Mariano; Habener, Joel F. (Lab. Molecular Endocrinol., Massachusetts Gen. Hosp., Harvard Med. Sch., Howard Hughes Med. Inst., Boston, MA, 02114, USA). Journal of Biological Chemistry, 272(31), 19562-19568 (English) 1997. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB We report the identification of the large subunit of the DNA replication factor, DSEB/RF-C140, as a new substrate for caspase-3 (**CPP32** /YAMA), or a very closely related protease activated during Fas-induced apoptosis in Jurkat T cells. DSEB/RF-C140 is a multifunctional DNA-binding protein with sequence homol. to poly(ADP-ribose) polymerase (PARP). This similarity includes a consensus DEVD/G cleavage site for caspase-3. Cleavage of DSEB/RF-C140 is predicted to occurs between Asp7-6 and Gly707, generating 87-kDa and 53-kDa fragments. An antiserum raised against the amino-terminal domain of DSEB/RF-C140 detects a new 87-kDa protein in Jurkat T cells in which apoptosis is activated by a monoclonal **antibody** to Fas. This cleavage occurs shortly after PARP cleavage. In vitro translated DSEB/RF-C140 is specifically cleaved into the predicted fragments when incubated with a cytoplasmic ext. from Fas **antibody**-treated cells. Proteolytic cleavage was prevented by substituting Asp706 by an alanine in the DEVD706/G caspase-3 cleavage site. The cleavage of DSEB/RF-C140 is prevented by iodoacetamide and the specific caspase-3 inhibitor, tetrapeptide aldehyde Ac-DEVD-CHO, but not by the specific ICE (interleukin-1-converting enzyme) inhibitors: CrmA and Ac-YVAD-CHO, indicating that the protease responsible for the cleavage of DSEB/RF-C140 during Fas-induced apoptosis in Jurkat cells is caspase-3, or a closely related protease. This conclusion is reinforced by the fact that recombinant caspase-3 but not caspase-1 reproduced the "in vitro" cleavage. Inasmuch as the cleavage of DSEB/RF-C140 separates its DNA binding from its assocn. domain, required for replication complex formation, we propose that such a cleavage will impair DNA replication. Recent in vitro mutagenesis support this proposal.

L9 ANSWER 15 OF 35 MEDLINE on STN DUPLICATE 4

97341172 Document Number: 97341172. PubMed ID: 9195941. Interferon-gamma modulates a p53-independent apoptotic pathway and apoptosis-related gene expression. Ossina N K; Cannas A; Powers V C; Fitzpatrick P A; Knight J D; Gilbert J R; Shekhtman E M; Tomei L D; Umansky S R; Kiefer M C. (LXR Biotechnology Inc., Richmond, California 94804, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 Jun 27) 272 (26) 16351-7. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Interferon (IFN)-gamma increases the sensitivity of tumor cell lines, many

of which are p53 mutants, to tumor necrosis factor-alpha-mediated and anti-Fas **antibody**-mediated cell death. To better understand the mechanism of IFN-gamma action in modulating the cell death response independently of p53 function, we analyzed the death of the human colon adenocarcinoma cell line, HT-29, following treatment with IFN-gamma and various cytotoxic agents. Here we show that IFN-gamma modulates cell death by sensitizing the cells to killing by numerous pro-apoptotic stimuli but not pro-necrotic stimuli. Furthermore, we show that select genes from several important apoptosis-related gene families are induced by IFN-gamma, including the apoptosis-signaling receptors CD95 (Fas/APO-1) and TNFR 1 and interleukin-1beta-converting enzyme (Ice) family members Ice, **CPP32** (Yama, **apopain**), ICErel-II (TX, Ich-2), Mch-3 (ICE-LAP3, CMH-1), Mch-4, and Mch-5 (MACH, FLICE). Of the bcl-2 family members, IFN-gamma directly induced bak but notably not bax, which is activated by p53. The IFN-responsive transcriptional activator interferon regulatory factor-1 was also strongly induced and translocated into the nucleus following IFN-gamma treatment. We propose that IFN-gamma modulates a p53-independent apoptotic pathway by both directly and indirectly inducing select apoptosis-related genes.

L9 ANSWER 16 OF 35 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 5

97116165 EMBASE Document No.: 1997116165. Selective activation of caspases during apoptotic induction in HL-60 cells. Effects of a tetrapeptide inhibitor. Polverino A.J.; Patterson S.D.. S.D. Patterson, Amgen Inc., Amgen Center, Mail Stop 14-2-E, 1840 DeHavilland Dr., Thousand Oaks, CA 91320-1789, United States. spatters@amgen.com. Journal of Biological Chemistry 272/11 (7013-7021) 1997.
Refs: 45.
ISSN: 0021-9258. CODEN: JBCHA3. Pub. Country: United States. Language: English. Summary Language: English.

AB Apoptosis is a highly regulated biochemical process that results in the selective death of cells. Members of the caspase family of cysteine proteases play a pivotal role in the effector phase of apoptosis. We show that, in HL- 60 cells, the addition of either anisomycin, a protein synthesis inhibitor, or geranylgeraniol, an intermediate in the cholesterol biosynthetic pathway, results in a rapid and en masse induction of apoptosis. The levels of actin, p42 and p44 MAPK, JNK1, JNK2, p38, and PCNA were not substantially altered during this process. Although these treatments appear to function by diverse pathways, they both result in the processing and activation of caspase-3 (**CPP32**.beta./Yama/ **Apopain**). In contrast, no activation of caspase-1 (interleukin-1.beta. converting enzyme (ICE)) was observed. Furthermore, we obtained ambiguous results regarding the activation of caspase-2 (Ich-1) depending on the **antibody** used. Pretreatment of the cells with benzyloxycarbonyl-Val-Ala-Asp- (OMe) -fluoromethylketone (zVAD.fmk), a tetrapeptide inhibitor of caspases, prevented the induction of apoptosis for 24 h. Even after 72 h of treatment, some cells were still alive and progressing through the cell cycle, suggesting that blockage of caspase activity is able to protect cells. These results suggest that selective activation of some caspases is necessary to induce apoptosis in HL-60 cells.

L9 ANSWER 17 OF 35 CAPLUS COPYRIGHT 2003 ACS on STN

1997:374447 Document No. 127:160549 Activation of protein kinase C attenuates early signals in Fas-mediated apoptosis. Del Carmen Ruiz-Ruiz, Maria; Izquierdo, Manuel; De Murcia, Gilbert; Lopez-Rivas, Abelardo (Instituto Parasitologia Biomedicina, Granada, E-18001, Spain). European Journal of Immunology, 27(6), 1442-1450 (English) 1997. CODEN: EJIMAF. ISSN: 0014-2980. Publisher: Wiley-VCH.

AB Activation of protein kinase C (PKC) was reported to inhibit Fas (APO-1, CD95) -mediated apoptosis in different cellular systems. Human Jurkat leukemic T cells express the Fas antigen in the cell membrane and undergo apoptosis upon crosslinking by anti-Fas monoclonal **antibodies** (mAb). Cleavage of the apoptosis-assocd. protease **CPP32** and its

substrate poly(ADP-ribose)polymerase are obsd. after the engagement of Fas antigen with mAb. All these effects are substantially inhibited by the activation of PKC with a phorbol ester. Bisindolylmaleimide, an inhibitor of PKC, prevents phorbol ester-induced down-regulation of Fas signaling. Inhibition of Fas-mediated cell death by phorbol ester is also obsd. in other human leukemic T cell lines. Crosslinking of Fas antigen by mAb results in the rapid increase in Tyr phosphorylation of several protein substrates which is further elevated in the presence of the protein Tyr phosphatase inhibitor, orthovanadate. Orthovanadate markedly enhances the cell death response to Fas mAb in different human leukemic T cell lines and human T cell blasts. These effects of orthovanadate on early Tyr phosphorylation and cell death are clearly diminished by PKC activation. These results strongly suggest that Tyr phosphorylation is involved in Fas signaling in apoptosis and that PKC plays a neg. role in Fas-mediated apoptosis by counteracting at a very early stage the signals generated following crosslinking of this receptor.

L9 ANSWER 18 OF 35 MEDLINE on STN DUPLICATE 6

1998100174 Document Number: 98100174. PubMed ID: 9437520. Disulfiram is a potent inhibitor of proteases of the caspase family. Nobel C S; Kimland M; Nicholson D W; Orrenius S; Slater A F. (Institute of Environmental Medicine, Division of Toxicology, Karolinska Institutet, Stockholm, Sweden.) CHEMICAL RESEARCH IN TOXICOLOGY, (1997 Dec) 10 (12) 1319-24. Journal code: 8807448. ISSN: 0893-228X. Pub. country: United States. Language: English.

AB We have recently shown that dithiocarbamate (DC) disulfides inhibit proteolytic processing of the caspase-3 proenzyme in Jurkat T lymphocytes treated with anti-CD95 (Fas/APO-1) **antibody**. Because the processing can be accomplished by caspase activity, we investigated the effect of DC disulfides, such as disulfiram (DSF), on active caspases. DSF showed a dose-dependent inhibition was prevented by including dithiothreitol (DTT) in the reaction buffer, thiol-disulfide exchange between inhibitor and target is suggested. Direct interaction of DSF with caspases was confirmed by its inhibition of the purified Ac-DEVD-AMC cleaving protease, caspase-3 (**CPP32/apopain**). An apparent rate constant (K(app)) for this inhibition was estimated to be 0.45 x 10(3)M(-1)s(-1). DSF was also observed to inhibit the purified Ac-YVAD-AMC cleaving enzyme, caspase-1 (interleukin-1 beta-converting enzyme, ICE), with a K(app) of 2.2 x 10(3) M(-1)s(-1). In this case protein mixed disulfide formation between DSF and caspase-1 was directly demonstrated using 35S-labeled DSF. The physiological disulfide GSSG was also observed to influence the activity of caspases. A glutathione buffer (5 mM) with a GSH:GSSG ratio of 9:1 decreased the Ac-DEVD-AMC cleaving activity in S100 cytosolic extracts by 50% as compared to GSH controls without GSSG. In conclusion, our study shows that caspases are quite sensitive to thiol oxidation and that DSF is a very potent oxidant of caspase protein thiol(s), being 700-fold more potent than glutathione disulfide.

L9 ANSWER 19 OF 35 MEDLINE on STN DUPLICATE 7

1998065786 Document Number: 98065786. PubMed ID: 9403528. Dysregulated expression of neutrophil apoptosis in the systemic inflammatory response syndrome. Jimenez M F; Watson R W; Parodo J; Evans D; Foster D; Steinberg M; Rotstein O D; Marshall J C. (Department of Surgery, University of Toronto, Toronto Hospital, Ontario, Canada.) ARCHIVES OF SURGERY, (1997 Dec) 132 (12) 1263-9; discussion 1269-70. Journal code: 9716528. ISSN: 0004-0010. Pub. country: United States. Language: English.

AB OBJECTIVE: To study the effect of the systemic inflammatory response syndrome (SIRS) or major elective surgery on the apoptosis of circulating polymorphonuclear neutrophils because an activated inflammatory response is terminated, in part, through the programmed cell death, or apoptosis, of its effector cells. DESIGN: A prospective inception cohort study. SETTING: A mixed surgical and medical intensive care unit of an adult tertiary care hospital. PATIENTS: Sixteen patients with SIRS, 7 uninfected patients who had undergone elective aortic aneurysmectomy, and

8 healthy laboratory control subjects. INTERVENTIONS: Serial blood samples were drawn for evaluation of neutrophil apoptosis, activational state, and surface receptor expression by flow cytometry. MAIN OUTCOME MEASURES: Spontaneous apoptosis was significantly delayed in neutrophils from patients with SIRS (8.6%+/-6.8%) and patients who had undergone elective aortic aneurysmectomy (11.0%+/-5.0%) when compared with controls (34.9%+/-6.8%). These neutrophils were activated as evidenced by enhanced respiratory burst activity and augmented surface expression of CD11b. Apoptosis in response to engagement of cell surface Fas (also known as CD95 or APO-1) with an agonistic **antibody** was blunted. Plasma from patients with SIRS or patients who had undergone elective aortic aneurysmectomy suppressed the apoptotic responses of control neutrophils (plasma from patients with SIRS, 18.8%+/-10.3%; plasma from patients who had undergone elective aortic aneurysmectomy, 20.0%+/-6.1%; P<.01). Western blot analysis showed normal expression of the key proapoptotic proteases, interleukin 1beta converting enzyme and **CPP32** (also known as YAMA, **apopain**, and caspase 3), indicating that delayed apoptosis was not a consequence of decreased levels of proapoptotic enzymes. CONCLUSIONS: Circulating neutrophils from patients with SIRS or from patients who have undergone major elective surgery show delayed expression of constitutive programmed cell death, and antiapoptotic factors are present in the general circulation. While prolonged neutrophil survival may represent an appropriate adaptive response to injury, the presence of activated and apoptosis-resistant cells in an antiapoptotic environment may contribute to the systemic inflammatory injury characteristic of SIRS and predispose to the development of the multiple organ dysfunction syndrome.

L9 ANSWER 20 OF 35 CAPLUS COPYRIGHT 2003 ACS on STN

1997:197128 Document No. 126:271906 Bcl-xL overexpression inhibits progression of molecular events leading to paclitaxel-induced apoptosis of human acute myeloid leukemia HL-60 cells. Ibrado, Ana Maria; Liu, Linda; Bhalla, Kapil (Division of Hematology/Oncology, Department of Medicine, Winship Cancer Center, Emory University School of Medicine, Atlanta, GA, 30322, USA). Cancer Research, 57(6), 1109-1115 (English) 1997. CODEN: CNREA8. ISSN: 0008-5472. Publisher: American Association for Cancer Research.

AB Paclitaxel has been shown to activate Raf-1 and cause phosphorylation of Bcl-2, which has been correlated with paclitaxel-induced apoptosis of cancer cells. In the present studies, the authors demonstrate that in human AML HL-60 cells that express Bcl-2 but little Bcl-xL (HL-60/neo cells), paclitaxel-induced phosphorylation of Bcl-2 is followed by increased intracellular free Bax levels. This, in turn, is followed by the cleavage and activation of the key cysteine protease, **CPP32** .beta./Yama, and cleavage of poly(ADP-ribose) polymerase, resulting in the DNA fragmentation of apoptosis. Cotreatment with the benzoquinone ansamycin Geldanamycin depleted Raf-1 but did not decrease Bcl-2 levels or impair paclitaxel-induced Bcl-2 phosphorylation in HL-60/neo cells. Also, Geldanamycin did not affect paclitaxel-induced apoptosis of HL-60/neo cells. As compared to the control HL-60/neo, HL-60/Bcl-xL cells contain Bcl-2 as well as an enforced overexpression of Bcl-xL. Immunopptn. studies with anti-Bcl-2 and/or anti-Bcl-x **antibodies** demonstrated that HL-60/Bcl-xL cells possess lower free Bax but higher levels of Bax heterodimerized to Bcl-2 and Bcl-xL. Following treatment of HL-60/Bcl-xL cells with paclitaxel, although Bcl-2 phosphorylation was obsd., it was not followed by increased free Bax levels, cleavage of **CPP32**.beta./Yama and poly(ADP-ribose) polymerase, or induction of the DNA fragmentation of apoptosis. These findings indicate the order of mol. events leading to paclitaxel-induced apoptosis and show that Raf-1 may not be involved in paclitaxel-induced phosphorylation of Bcl-2 or apoptosis of HL-60 cells.

L9 ANSWER 21 OF 35 MEDLINE on STN

97224067 Document Number: 97224067. PubMed ID: 9070648. Actin cleavage by CPP-32/**apopain** during the development of apoptosis. Mashima T;

Naito M; Noguchi K; Miller D K; Nicholson D W; Tsuruo T. (Laboratory of Biomedical Research, Institute of Molecular and Cellular Biosciences, University of Tokyo, Japan.) ONCOGENE, (1997 Mar 6) 14 (9) 1007-12. Journal code: 8711562. ISSN: 0950-9232. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Interleukin-1beta-converting enzyme (ICE)/ced-3 family proteases play key roles in apoptosis. However, cellular substrates for ICE family proteases involved in apoptosis are not well understood. We previously showed that actin is cleaved in vitro by an ICE family protease, distinct from ICE itself, which is activated during VP-16-induced apoptosis. In this report, we demonstrate that the actin-cleaving ICE-family protease in the apoptotic cell extract is the activated CPP-32/**apopain**. CPP-32 effectively cleaves actin protein to 15 kDa and 31 kDa fragments. Studies with an **antibody** raised against Gly-Gln-Val-Ile-Thr peptide, the N-terminal sequence of the cleaved 15 kDa actin fragment, showed that actin is also cleaved in vivo during the development of apoptosis. Moreover, Benzyloxycarbonyl-Glu-Val-Asp-CH2OC(O)-2,6,-dichlorobenzene (Z-EVD-CH2-DCB), a selective inhibitor of CPP-32(-like) protease, efficiently inhibited the cleavage of actin and the apoptosis of VP-16-treated U937 cells. Our present results indicate that actin is the substrate of CPP-32/**apopain**(-like) protease both in vitro and in vivo and suggest the role of actin in the control of cell growth and apoptosis.

L9 ANSWER 22 OF 35 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

97368496 EMBASE Document No.: 1997368496. Induction of apoptosis by cryptophycin 1, a new antimicrotubule agent. Mooberry S.L.; Busquets L.; Tien G.. S.L. Mooberry, Cancer Research Center of Hawaii, 1236 Lauhala Street, Honolulu, HI 96813, United States. sue@crch.hawaii.edu. International Journal of Cancer 73/3 (440-448) 1997.
Refs: 26.
ISSN: 0020-7136. CODEN: IJCNAW. Pub. Country: United States. Language: English. Summary Language: English.

AB The ability of cryptophycin I, a new potent cytotoxic antimicrotubule agent, to initiate apoptosis was studied. Treatment of cells with cryptophycin I (50 pM) rapidly caused morphological changes consistent with the induction of apoptosis. DNA strand breakage and fragmentation of the DNA into oligonucleosome-sized fragments was observed, and this coincided with the loss of cellular DNA. Activation of the cysteine protease **CPP32** (caspase 3, YAMA, **apopain**), a member of the ICE/CED-3-like protease family of apoptosis effectors, was consistent with the execution of cell death by a coordinated sequence of events. Low concentrations of cryptophycin I caused mitotic arrest with the formation of abnormal mitotic spindles without affecting interphase microtubule structures. Unlike other microtubule active agents, cryptophycin-induced mitotic arrest persisted for only a brief period before the onset of apoptosis. There was no evidence of release from G2/M cell cycle arrest. Our results show that low concentrations of cryptophycin I (50 pM) initiated cell death consistent with apoptosis. These data suggest that the cytotoxic effects of cryptophycin I are due in part to its ability to initiate apoptosis rapidly.

L9 ANSWER 23 OF 35 CAPLUS COPYRIGHT 2003 ACS on STN

1997:293115 Document No. 127:3459 Aggregation of Thy-1 glycoprotein induces thymocyte apoptosis through activation of **CPP32**-like proteases. Fujita, Naoya; Kodama, Nobuyuki; Kato, Yukinari; Lee, Sang-Han; Tsuruo, Takashi (Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo, 113, Japan). Experimental Cell Research, 232(2), 400-406 (English) 1997. CODEN: ECREAL. ISSN: 0014-4827. Publisher: Academic.

AB Mouse thymocytes are known to undergo apoptosis by ligating some unique anti-Thy-1 monoclonal **antibodi s** (mAbs), G7 and KT16. However, the precise mechanisms of Thy-1-mediated apoptosis are as yet unclear. We investigated Thy-1-mediated apoptosis using our previously generated anti-Thy-1 mAb, MCS-34, which was similar to G7 because both

antibodies recognized both Thy-1.1 and Thy-1.2 and bound Thy-1A epitope. Unlike G7, MCS-34 alone could not induce apoptosis in thymocytes; however, it could induce apoptosis when it was cross-linked with second **antibodies**. Thus, MCS-34 could not aggregate by itself, but G7 could. In the course of investigating the apoptosis-related mols. that were involved in the thymocyte apoptosis induced by crosslinking of MCS-34 or by G7 ligation, we found that **CPP32**-like proteases were activated during the apoptosis. Furthermore, the expression of bcl-2 and bcl-XL proteins was decreased in these apoptosis processes. Whereas the ligation of MCS-34 alone could not generate apoptosis signals that led to the activation of **CPP32** -like proteases and the decrease in bcl-2 and bcl-XL expression, the aggregation of Thy-1 glycoprotein might be crucial to signal thymocyte apoptosis. These results indicate that MCS-34 is a useful anti-Thy-1 mAb for analyzing the Thy-1-mediated signals since MCS-34 can control the level of apoptosis by using second **antibodies**.

L9 ANSWER 24 OF 35 MEDLINE on STN DUPLICATE 8

97312366 Document Number: 97312366. PubMed ID: 9168807. Intact cell evidence for the early synthesis, and subsequent late **apopain** -mediated suppression, of poly(ADP-ribose) during apoptosis. Rosenthal D S; Ding R; Simbulan-Rosenthal C M; Vaillancourt J P; Nicholson D W; Smulson M. (Department of Biochemistry and Molecular Biology, Georgetown University School of Medicine, Washington, DC 20007, USA.) EXPERIMENTAL CELL RESEARCH, (1997 May 1) 232 (2) 313-21. Journal code: 0373226. ISSN: 0014-4827. Pub. country: United States. Language: English.

AB Poly(ADP-ribose) polymerase (PARP), which is catalytically activated by DNA strand breaks, has been implicated in apoptosis, or programmed cell death. A protease (**CPP32**) responsible for the cleavage of PARP and necessary for apoptosis was recently purified and characterized. The coordinated sequence of events related to PARP activation and cleavage in apoptosis has now been examined in individual cells. Apoptosis was studied in a human osteosarcoma cell line that undergoes a slow (8 to 10 days), spontaneous, and reproducible death program in culture. Changes in the abundance of intact PARP, poly(ADP-ribose) (PAR), and a proteolytic cleavage product of PARP that contains the DNA-binding domain were examined during apoptosis in the context of individual, whole cells by immunofluorescence with specific **antibodies**. The synthesis of PAR from NAD increased early, within 2 days of cell plating for apoptosis, prior to the appearance of internucleosomal DNA cleavage and before the cells become irreversibly committed to apoptosis, since replating yields viable, nonapoptotic cells. Strong expression of full-length PARP was also detected, by immunofluorescence as well as by Western analysis, during this same time period. However, after approximately 4 days in culture, the abundance of both full-length PARP and PAR decreased markedly. After 6 days, a proteolytic cleavage product containing the DNA-binding domain of PARP was detected immunocytochemically and confirmed by Western analysis, both in the nuclei and in the cytoplasm of cells. A recombinant peptide spanning the DNA-binding domain of PARP was expressed, purified, and biotinylated, and was then used as a probe for DNA strand breaks. Fluorescence microscopy with this probe revealed extensive DNA fragmentation during the later stages of apoptosis. This is the first report, using individual, intact cells, demonstrating that poly(ADP-ribosyl)ation of nuclear proteins occurs prior to the commitment to apoptosis, that inactivation and cleavage of PARP begin shortly thereafter, and that very little PAR per se is present during the later stages of apoptosis, despite the presence of a very large number of DNA strand breaks. These results suggest a negative regulatory role for PARP during apoptosis, which in turn may reflect the requirement for adequate NAD and ATP during the later stages of programmed cell death.

L9 ANSWER 25 OF 35 MEDLINE on STN DUPLICATE 9

97376992 Document Number: 97376992. PubMed ID: 9233763. Involvement of caspase-4(-like) protease in Fas-mediated apoptotic pathway. Kamada S; Washida M; Hasegawa J; Kusano H; Funahashi Y; Tsujimoto Y. (Department of

Medical Genetics, Biomedical Research Center, Osaka University Medical School, Suita, Japan.) ONCOGENE, (1997 Jul 17) 15 (3) 285-90. Journal code: 8711562. ISSN: 0950-9232. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Proteases of the caspase family, especially caspase-1 (ICE)(-like), caspase-3 (**CPP32**/Yama/**apopain**)(-like) and caspase-8 (MACH/FLICE/Mch5) proteases, are implicated in Fas (APO-1/CD95)-mediated apoptosis. Here, we show that the caspase-4 (TX/ICH-2/ICE(rel)II)(-like) protease, another member of the caspase family, is also involved in Fas-mediated apoptosis, based upon the observations: (i) caspase-4 is processed in response to an agonistic anti-Fas **antibody** treatment, (ii) overexpression of a mutant caspase-4 with active site mutations in both p20 and p10 subunits delays Fas-mediated apoptosis, (iii) microinjected anti-caspase-4 **antibodies** inhibit Fas-mediated apoptosis. Together with our observations that the mutant caspase-4 inhibits the Fas-mediated activation of caspase-3(-like) proteases and purified caspase-4 cleaves pro-caspase-3 to generate a subunit of active form, these results suggest that Fas-mediated apoptosis is driven by a caspase cascade in which the caspase-4(-like) protease transmits a death signal from caspase-8 to caspase-3(-like) proteases probably through directly cleaving pro-caspase-3(-like) proteases.

L9 ANSWER 26 OF 35 CAPLUS COPYRIGHT 2003 ACS on STN

1996:746318 Document No. 126:16248 Purification and characterization of the human pro-apoptotic cysteine proteinase, **apopain**, and its modulation by peptidyl inhibitors or gene therapy. Miller, Douglas K.; Thornberry, Nancy A.; Nicholson, Donald W.; Ali, Ambereen; Vaillancourt, John P. (Merck and Co., Inc., USA; Merck Frosst Canada Inc.; Miller, Douglas K.; Thornberry, Nancy A.; Nicholson, Donald W.; Ali, Ambereen; Vaillancourt, John P.). PCT Int. Appl. WO 9633268 A1 19961024, 83 pp. DESIGNATED STATES: W: CA, JP, US; RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE. (English). CODEN: PIXXD2. APPLICATION: WO 1996-US5282 19960417. PRIORITY: US 1995-426557 19950421.

AB The present invention is directed to an isolated and purified enzyme designated **apopain**, methods of using **apopain** to screen for compds. which modulate the activity of **apopain**, and compds. identified by the screens. Thus, a poly(ADP-ribose) polymerase cleavage activity (**apopain**) was detected in progressively apoptotic human osteosarcoma cells. **Apopain** was purified and structural anal. showed it to comprise 2 subunits (p17 and p12) proteolytically processed from the 32-kDa **CPP32** precursor by cleavage at the Asp28-Ser29 and Asp175-Ser176 positions. A synthetic DNA mol. encoding full-length **apopain** is prepd. from the purified enzyme. The synthetic **apopain**-encoding DNA is formulated so as to optimize expression in a variety of recombinant hosts. The DNA clones produce recombinant full-length **apopain** and derivs. thereof. Purified native **apopain** and recombinant **apopain** are useful for identifying modulators of **apopain** activity and hence modifier of pathol. conditions related to the pro-inflammatory or pro-apoptotic effects of **apopain**. Thus, the tetrapeptide aldehyde inhibitor Ac-YVAD-CHO acts with a Ki of <1 nM, making it among the most potent peptide aldehydes known for a cysteine proteinase. The synthesis of Ac-YVAD-CHO is described in detail, and can be generally applied for the synthesis of other peptidyl inhibitors. **Apopain** antisense mols. are useful for therapeutically reducing or eliminating the pro-inflammatory or pro-apoptotic effects of **apopain**, whereas gene transplantation or gene therapy with **apopain** is useful for enhancing the pro-inflammatory or pro-apoptotic effects of **apopain**. These therapies are beneficial in the treatment of immune, proliferative and degenerative diseases including, but not limited to, immune deficiency syndromes (such as AIDS), autoimmune diseases, pathogenic infections, cardiovascular and neurol. injury, alopecia, aging, cancer, Parkinson's disease and Alzheimer's disease.

L9 ANSWER 27 OF 35 MEDLINE on STN DUPLICATE 10

96212185 Document Number: 96212185. PubMed ID: 8626669. D4-GDI, a substrate of **CPP32**, is proteolyzed during Fas-induced apoptosis. Na S; Chuang T H; Cunningham A; Turi T G; Hanke J H; Bokoch G M; Danley D E. (Department of Molecular Sciences, Pfizer Inc., Groton, Connecticut 06340, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 May 10) 271 (19) 11209-13. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Apoptosis (programmed cell death) is a fundamental process for normal development of multicellular organisms, and is involved in the regulation of the immune system, normal morphogenesis, and maintenance of homeostasis, ICE/CED-3 family cysteine proteases have been implicated directly in apoptosis, but relatively few of the substrates through which their action is mediated have been identified. Here we report that D4-GDI, an abundant hematopoietic cell GDP dissociation inhibitor for the Ras-related Rho family GTPases, is a substrate of the apoptosis protease **CPP32**/Yama/**Apopain**. D4-GDI was rapidly truncated to a 23-kDa fragment in Jurkat cells with kinetics that parallel the onset of apoptosis following Fas cross-linking with agonistic **antibody** or treatment with staurosporine. Fas- and staurosporine-induced apoptosis as well as cleavage of D4-GDI were inhibited by the ICE inhibitor, YVAD-cmk. D4-GDI was cleaved in vitro by recombinant **CPP32** expressed in Escherichia coli to form a 23-kDa fragment. The **CPP32**-mediated cleavage of D4-GDI was completely inhibited by 1 microM DEVD-CHO, a reported selective inhibitor of **CPP32**. In contrast, the ICE-selective inhibitors, YVAD-CHO or YVAD-cmk, did not inhibit **CPP32**-mediated D4-GDI cleavage at concentrations up to 50 microM. N-terminal sequencing of the 23-kDa D4-GDI fragment demonstrated that D4-GDI was cleaved between Asp19 and Ser20 of the poly(ADP-ribose) polymerase-like cleavage sequence DELD19S. These data suggest that regulation by D4-GDI of Rho family GTPases may be disrupted during apoptosis by **CPP32**-mediated cleavage of the GDI protein.

L9 ANSWER 28 OF 35 MEDLINE on STN

96214865 Document Number: 96214865. PubMed ID: 8617712. Molecular ordering of the cell death pathway. Bcl-2 and Bcl-xL function upstream of the CED-3-like apoptotic proteases. Chinnaiyan A M; Orth K; O'Rourke K; Duan H; Poirier G G; Dixit V M. (Department of Pathology, University of Michigan Medical School, Ann Arbor 48109, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Mar 1) 271 (9) 4573-6. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Genetic analyses of Caenorhabditis elegans has identified three genes that function in the regulation of nematode cell death. Mammalian homologs of two of these genes, ced-9 and ced-3, have been identified and comprise proteins belonging to the Bcl-2 and ICE families, respectively. To date, it is unclear where the negative regulators, ced-9 and bcl-2, function relative to the death effectors, ced-3 and the mammalian ced-3 homologs, respectively. Here, the molecular order of the cell death pathway is defined. Our results establish that Bcl-2 and Bcl-xL function upstream of two members of the ICE/CED-3 family of cysteine proteases, Yama (**CPP32/apopain**) and ICE-LAP3 (Mch3).

L9 ANSWER 29 OF 35 CAPLUS COPYRIGHT 2003 ACS on STN

1996:649120 Document No. 125:299361 Requirement of p34cdc2 kinase for apoptosis mediated by the Fas/APO-1 receptor and interleukin 1.beta.-converting enzyme-related proteases. Yao, Siu-Long; McKenna, Karen A.; Sharkis, Saul J.; Bedi, Atul (Johns Hopkins Oncology Center, Johns Hopkins Univ. School Medicine, Baltimore, MD, 21287, USA). Cancer Research, 56(20), 4551-4555 (English) 1996. CODEN: CNREA8. ISSN: 0008-5472. Publisher: American Association for Cancer Research.

AB The induction of apoptosis by the Fas/APO-1 receptor is important for T-cell-mediated cytotoxicity and down-regulation of immune responses. Binding of Fas ligand to the Fas/APO-1 receptor transduces an apoptotic signal that requires activation of interleukin 1.beta.-converting enzyme (ICE) and **CPP32**.beta. (**Apopain**/Yama), members of a family of cysteine proteases that are evolutionarily conserved

determinants of cell death. We report here that Fas/APO-1-triggered apoptosis involves ICE-mediated activation of p34cdc2 kinase. Ligation of the Fas receptor resulted in the rapid stimulation of ICE proteolytic activity and activation of p34cdc2 kinase. Specific tetrapeptide inhibitors of ICE (Acetyl-Tyr-Val-Ala-Asp-chloromethylketone) or of **CPP32**.beta. (Acetyl-Asp-Glu-Val-Asp-aldehyde) prevented the anti-Fas **antibody**-mediated activation of p34cdc2 and inhibited apoptosis. Inhibition of p34cdc2 activity by transient overexpression of a dominant-neg. cdc2 construct or human WEE1 kinase inhibited Fas-mediated apoptosis. These results suggest that activation of p34cdc2 kinase is a crit. determinant of cell death mediated by Fas and ICE family proteases.

L9 ANSWER 30 OF 35 CAPLUS COPYRIGHT 2003 ACS on STN
1997:3486 Document No. 126:116156 Proteolytic activation of protein kinase C .delta. by an ICE/CED 3-like protease induces characteristics of apoptosis. Ghayur, Tariq; Hugunin, Margaret; Talanian, Robert V.; Ratnofsky, Sheldon; Quinlan, Christopher; Emoto, Yutaka; Pandey, Pramod; Datta, Rakesh; Huang, Yinyin; Kharbanda, Surender; Allen, Hamish; Kamen, Robert; Wong, Winnie; Kufe, Donald (BASF Bioresearch Corporation, Worcester, MA, 01605, USA). Journal of Experimental Medicine, 184(6), 2399-2404 (English) 1996. CODEN: JEMEAV. ISSN: 0022-1007. Publisher: Rockefeller University Press.

AB Recent studies have shown that protein kinase C (PKC) .delta. is proteolytically activated at the onset of apoptosis induced by DNA-damaging agents, tumor necrosis factor, and anti-Fas **antibody**. However, the relation of PKC.delta. cleavage to induction of apoptosis is unknown. The present studies demonstrate that full-length PKC.delta. is cleaved at DMQD330N to a catalytically active fragment by the cysteine protease **CPP32**. The results also demonstrate that overexpression of the catalytic kinase fragment in cells is assocd. with chromatin condensation, nuclear fragmentation, induction of sub-G1 phase DNA and lethality. By contrast, overexpression of full-length PKC.delta. or a kinase inactive PKC.delta. fragment had no detectable effect. The findings suggest that proteolytic activation of PKC.delta. by a **CPP32**-like protease contributes to phenotypic changes assocd. with apoptosis.

L9 ANSWER 31 OF 35 MEDLINE on STN DUPLICATE 11
96147148 Document Number: 96147148. PubMed ID: 8567626. **CPP32**/**apopain** is a key interleukin 1 beta converting enzyme-like protease involved in Fas-mediated apoptosis. Schlegel J; Peters I; Orrenius S; Miller D K; Thornberry N A; Yamin T T; Nicholson D W. (Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Jan 26) 271 (4) 1841-4. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Cysteine proteases of the interleukin 1 beta Converting Enzyme (ICE)/CED-3 family have been implicated in the effector process of apoptosis in several systems, including Fas-mediated apoptosis. We have recently isolated and partially characterized a protease present in extracts from anti-Fas **antibody** treated Jurkat T cells that promotes apoptotic changes in isolated nuclei (Schlegel, J., Peters, I., and Orrenius, S. (1995) FEBS Lett. 364, 139-142). We now show that this protease cleaves poly-(ADP-ribose) polymerase (PARP) with high efficiency and specificity. Both PARP proteolysis and the proapoptotic effects of the protease are inhibited by nanomolar concentrations of a selective inhibitor of **apopain** (**CPP32**), while an inhibitor of IL-1 beta converting enzyme is much less effective, requiring micromolar concentrations for the inhibition of the isolated protease. Kinetic analysis of the isolated protease reveals kinetic constants similar to those reported for **apopain**. The isolated protease is recognized by **antibodies** specific for **CPP32**/**apopain** but not by an anti-ICE **antibody**. Furthermore, a selective inhibitor of **apopain** prevents Fas-induced apoptosis in intact Jurkat T cells. We therefore conclude that **CPP32**/**apopain** is

activated in Fas-induced apoptosis.

L9 ANSWER 32 OF 35 CAPLUS COPYRIGHT 2003 ACS on STN
1996:238695 Document No. 124:285689 Involvement of **CPP32** /Yama(-like) proteases in Fas-mediated apoptosis. Hasegawa, Jun-ichi; Kamada, Shinji; Kamlike, Wataru; Shimizu, Shigeomi; Imazu, Tetsuo; Matsuda, Hikaru; Tsujimoto, Yoshihide (Frist Department Surgery, Osaka University Medical School, Suita, 565, Japan). Cancer Research, 56(8), 1713-18 (English) 1996. CODEN: CNREA8. ISSN: 0008-5472. Publisher: American Association for Cancer Research.

AB Fas (Apo-1/CD95) belongs to the tumor necrosis factor/nerve growth factor receptor family and transmits apoptotic signals by binding to its ligand. Interleukin-1.beta.-converting enzyme (ICE), which shows substantial homol. to the product of the cell death gene, ced-3, of Caenorhabditis elegans, is reported to be involved in Fas-mediated apoptosis. Using two human carcinoma-derived cell lines with undetectable levels of ICE, the authors found that an agonistic anti-human Fas **antibody** induces the activation of **CPP32**/Yama(-like) proteases that are ICE(-like) protease family members, and that a tetrapeptide inhibitor of **CPP32**/Yama protease, DEVD-CHO, inhibits the Fas-mediated activation of the proteases, Fas-mediated apoptosis, and **CPP32** /Yama(-like) proteolytic activities in vitro. Fas-mediated apoptosis is inhibited by the **CPP32**/Yama inhibitor DEVD-CHO, but not by the ICE inhibitor YVAD-CHO, suggesting a dominant role for the **CPP32** /Yama(-like) proteases and not ICE itself in Fas-mediated apoptosis of the human carcinoma cell lines.

L9 ANSWER 33 OF 35 MEDLINE on STN DUPLICATE 12
96228046 Document Number: 96228046. PubMed ID: 8647264. Ligation of CD40 rescues Ramos-Burkitt lymphoma B cells from calcium ionophore- and antigen receptor-triggered apoptosis by inhibiting activation of the cysteine protease **CPP32**/Yama and cleavage of its substrate PARP. An S; Knox K A. (Department of Biochemistry, The University of Oxford, UK.) FEBS LETTERS, (1996 May 20) 386 (2-3) 115-22. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB The new and growing family of interleukin-1beta-converting enzyme (ICE) cysteine proteases are now recognised to be major effectors of cellular death by apoptosis. Like other members of this family, the **CPP32** /Yama proform is activated by processing to its active heterodimeric enzyme or **apopain** when it likely contributes to the process of apoptosis by cleaving poly(ADP-ribose) polymerase (PARP) and thereby inhibiting much of its DNA repair activity. Apoptosis plays a fundamental role in the regulation of the immune system where it is involved in the selection of both T and B lymphocytes bearing antigen receptor (AgR) for non-self. Cells of the Ramos Epstein-Barr virus (EBV)-genome-negative Burkitt lymphoma (BL) B cell line (Ramos-BL) can be triggered into growth arrest and apoptosis by treating with the calcium ionophore ionomycin or by crosslinking their surface AgR with **antibodies** directed against immunoglobulin (Ig)M (anti-IgM). Ionomycin- and AgR-triggered growth arrest and apoptosis are arrested by signals transduced through the surface CD40 of Ramos-BL B cells. Both ionomycin and anti-IgM trigger activation of **CPP32** and cleavage of PARP prior to the onset of apoptosis; this process is abrogated by treatment with anti-CD40 and is independent of Bcl-2 expression. A tripeptide inhibitor of ICE family cysteine proteases, Z-Val-Ala-Asp-fluoromethylketone (zVAD-fmk) inhibits ionomycin- and AgR-triggered **CPP32** activation, PARP cleavage and apoptosis, but not growth arrest, in Ramos-BL B cells. Thus, in this report we demonstrate that in a physiological system, activation of endogenous members of the ICE family, including **CPP32**, and cleavage of the death substrate PARP act as major effectors of apoptotic death.

L9 ANSWER 34 OF 35 MEDLINE on STN DUPLICATE 13
96292225 Document Number: 96292225. PubMed ID: 8700552. Chromosome 22 complements apoptosis in Fas-and TNF-resistant mutant UK110 cells. Noguchi

K; Naito M; Oshimura M; Mashima T; Fujita N; Yonehara S; Tsuruo T. (Laboratory of Biomedical Research, Institute of Molecular and Cellular Biosciences, University of Tokyo.) ONCOGENE, (1996 Jul 4) 13 (1) 39-46. Journal code: 8711562. ISSN: 0950-9232. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Fas and p55 tumor necrosis factor receptor (TNFR) transfer an apoptosis signal when they are crosstinked with their ligands or agonistic **antibodies**. However, the signal transduction mechanism of apoptosis via Fas and p55 TNFR has not yet been elucidated. We previously described a recessive mutant UK110 from the human monocytic leukemia U937 cell line, that showed resistance against Fas- and p55 TNFR-mediated apoptosis. By cytogenetic analysis and microcell-fusion method, we demonstrate here that introduction of chromosome 22 can specifically restore the sensitivity to Fas- and TNF-mediated apoptosis in UK110 cells. Moreover, introduction of chromosome 22 into UK110 can complement the processing of interleukin-1 beta converting enzyme (ICE)-like proteases, such as **CPP32**/Yama/**Apopain** and ICH-1L, after treatment with anti-Fas and anti-p55 TNFR **antibodies**. These results suggest that the product of a gene located on chromosome 22 participates in the Fas-and p55 TNFR-mediated apoptosis at a point upstream of ICE-like proteases.

L9 ANSWER 35 OF 35 MEDLINE on STN DUPLICATE 14

96397518 Document Number: 96397518. PubMed ID: 8804412. **CPP32** /Yama/**apopain** cleaves the catalytic component of DNA-dependent protein kinase in the holoenzyme. Teraoka H; Yumoto Y; Watanabe F; Tsukada K; Suwa A; Enari M; Nagata S. (Department of Pathological Biochemistry, Tokyo Medical and Dental University, Japan.. hteraoka.pbc@mri1.mri.tmd.ac.jp) . FEBS LETTERS, (1996 Sep 9) 393 (1) 1-6. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB DNA-dependent protein kinase (DNA-PK) is composed of a 460-kDa catalytic component (p460) and a DNA-binding component Ku protein. Immunoblot analysis after treatment of Jurkat cells with anti-Fas **antibody** demonstrated the cleavage of p460 concomitantly with an increase in **CPP32**/Yama/**apopain** activity. Recombinant **CPP32** /Yama/**apopain** specifically cleaved p460 in the DNA-PK preparation that had been purified from Raji cells into 230- and 160-kDa polypeptides, the latter of which was detected in anti-Fas-treated Jurkat cells. The regulatory component Ku protein was not significantly affected by **CPP32**/Yama/**apopain**. DNA-PK activity was decreased with the disappearance of p460 in the incubation of DNA-PK with **CPP32**/Yama/**apopain**. These results suggest that the catalytic component of DNA-PK is one of the target proteins for **CPP32**/Yama/**apopain** in Fas-mediated apoptosis.

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     FILE 'MEDLINE, EMBASE, BIOSIS, SCISEARCH, CAPLUS' ENTERED AT 09:29:32 ON
     20 OCT 2003
L1          1406 S RUBEN S?/AU
L2             5 S L1 AND ICE
L3             5 DUP REMOVE L2 (0 DUPLICATES REMOVED)
L4           527 S L1 AND ANTIBOD?
L5             1 S L4 AND "ICE LAP-6"
L6          1030 S ANTIBODY AND CPP32
L7            85 S L6 AND APOPAIN
L8             1 S L7 AND MCH2
L9            35 DUP REMOVE L7 (50 DUPLICATES REMOVED)

=> s caspase review
L10           16 CASPASE REVIEW
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=> dup remove l10
PROCESSING COMPLETED FOR L10
L11 16 DUP REMOVE L10 (0 DUPLICATES REMOVED)

=> d l11 1-16 cbib abs

L11 ANSWER 1 OF 16 CAPLUS COPYRIGHT 2003 ACS on STN
2003:326109 Document No. 139:114828 Mammalian mitochondrial IAP binding proteins. Vaux, David L.; Silke, John (The Walter and Eliza Hall Institute of Medical Research, Parkville, 3050, Australia). Biochemical and Biophysical Research Communications, 304(3), 499-504 (English) 2003. CODEN: BBRCA9. ISSN: 0006-291X. Publisher: Elsevier Science.
AB A review. Four mitochondrial proteins have been identified that immunoppt. with the mammalian inhibitor of apoptosis (IAP) protein XIAP. Each of them interacts via a processed amino terminus that resembles those of the insect pro-apoptotic IAP binding proteins Grim, HID, Reaper, and Sickle. Two, Diablo/Smac and HrtA2/Omi, have been extensively characterized. Both Diablo and HtrA2 can bind to IAPs and promote apoptosis when over-expressed in transfected cells, but unlike the insect IAP antagonists, to date there is scant evidence that they are important regulators of apoptosis in more physiol. circumstances.

L11 ANSWER 2 OF 16 CAPLUS COPYRIGHT 2003 ACS on STN
2001:113363 Document No. 135:207468 Key targets for the execution of radiation-induced tumor cell apoptosis: the role of p53 and caspases. Pruschy, M.; Rocha, S.; Zaugg, K.; Tenzer, A.; Hess, C.; Fisher, D. E.; Glanzmann, C.; Bodis, S. (Department of Radiation Oncology, University Hospital Zurich, Zurich, Switz.). International Journal of Radiation Oncology, Biology, Physics, 49(2), 561-567 (English) 2001. CODEN: IOBPD3. ISSN: 0360-3016. Publisher: Elsevier Science Inc..
AB A review with 48 refs. In many human hematol. and solid malignancies, intrinsic or acquired treatment resistance remains a major obstacle for successful cancer therapy. The mol. understanding of how tumor cells respond to chemotherapy and ionizing radiation is rapidly evolving. Induction of programmed cell death, apoptosis, is one important strategy for successful cancer therapy. This has been shown convincingly for oncogene-transformed normal cells as well as tumor cells of lymphoid origin. However, the relevance of apoptosis in solid human malignancies is less clear. Loss of apoptosis might be linked to specific mutations in the often tissue-specific apoptotic pathways due to aberrations in the stress-related signal transduction cascades. Restoration of a dysfunctional apoptotic program in cancer tissue where apoptosis has been identified as an important mechanism for tissue homeostasis is one rational approach for innovative cancer therapy. In this review, we focus on the relevance of the tumor suppressor p53 for apoptosis-induction and successful cancer therapy outlining the importance of an intact caspase machinery for apoptosis execution. Strategies are discussed to overcome treatment resistance and a high apoptotic threshold in human malignancies where apoptosis is the dominant mode of cell death and the status of p53 is an important determinant for apoptosis induction.

L11 ANSWER 3 OF 16 CAPLUS COPYRIGHT 2003 ACS on STN
2000:849908 Document No. 134:307346 Cytometry of caspases. Koester, Steven K.; Bolton, Wade E. (Advanced Technology, Beckman Coulter, Inc., Miami, FL, 33196, USA). Methods in Cell Biology, 63, 487-504 (English) 2001. CODEN: MCBLAG. ISSN: 0091-679X. Publisher: Academic Press.
AB A review with 44 refs. Topics discussed include caspase inhibition followed by CD95 induction of Jurkat cells; staining of cells for APO2.7 expression; staining of cells for viability status with antitubulin antibody; method for distinguishing viable, early apoptotic, late apoptotic, and necrotic cells by flow cytometry; annexin V staining for phosphatidylserine exposure; light scatter measurements by flow cytometry; detection of DNA fragments; staining of cells for viability status with trypan blue; ultrastructural anal. with TEM; and fluorogenic substrates for flow cytometric identification of caspase activation. (c) 2001

Academic Press.

L11 ANSWER 4 OF 16 CAPLUS COPYRIGHT 2003 ACS on STN
2002:375077 Document No. 137:199783 Autoimmune lymphoproliferative syndrome: Types I, II and beyond. Chun, Hyung J.; Lenardo, Michael J. (Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, 20892, USA). Advances in Experimental Medicine and Biology, 490(Mechanisms of Lymphocyte Activation and Immune Regulation VIII), 49-57 (English) 2001. CODEN: AEMBAP. ISSN: 0065-2598. Publisher: Kluwer Academic/Plenum Publishers.
AB A review. Autoimmune lymphoproliferative syndrome (ALPS) provides novel insights into mechanisms that regulate lymphocyte homeostasis and is assocd. with dominant-interfering mutations in 3 genes Fas receptor, Fas ligand and Caspase 10.

L11 ANSWER 5 OF 16 CAPLUS COPYRIGHT 2003 ACS on STN
2001:47642 Document No. 134:233115 More than one way to go. Wyllie, Andrew H.; Golstein, Pierre (Department of Pathology, University of Cambridge, Cambridge, CB2 1QP, UK). Proceedings of the National Academy of Sciences of the United States of America, 98(1), 11-13 (English) 2001. CODEN: PNASA6. ISSN: 0027-8424. Publisher: National Academy of Sciences.
AB A review, with 36 refs. Topics discussed include caspase-dependent (apoptosis) and -independent (paraptosis) cell death.

L11 ANSWER 6 OF 16 CAPLUS COPYRIGHT 2003 ACS on STN
2000:617214 Document No. 133:307951 The most unkindest cut of all: On the multiple roles of mammalian caspases. Fadeel, B.; Orrenius, S.; Zhivotovsky, B. (Division of Toxicology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, 171 77, Swed.). Leukemia, 14(8), 1514-1525 (English) 2000. CODEN: LEUKED. ISSN: 0887-6924. Publisher: Nature Publishing Group.
AB A review with 167 refs. The caspases, first discovered almost a decade ago, are intracellular cysteine proteases which have been shown to play an essential role in the initiation and execution phases of apoptotic cell death. Numerous strategies for the activation and inhibition of these "killer" proteases have evolved, including the regulation of caspase expression and function at the transcriptional and post-translational level, as well as the expression of viral and cellular inhibitors of caspases. Emerging evidence in recent years has also implicated the caspases in various, non-apoptotic aspects of cellular physiol., such as cytokine processing during inflammation, differentiation of progenitor cells during erythropoiesis and lens fiber development, and proliferation of T lymphocytes, thus attesting to the pleiotropic functions of these proteases. The present review aims to discuss the multiple roles of the mammalian caspases with particular emphasis on their activation and regulation in cells of leukemic origin and the attendant possibilities of therapeutic intervention.

L11 ANSWER 7 OF 16 CAPLUS COPYRIGHT 2003 ACS on STN
2000:568203 Document No. 134:276175 Transient transfection assay of cell death genes. Miura, Masayuki; Yuan, Junying (Department of Neuroanatomy, Osaka University Medical School, Osaka, 565-0871, Japan). Methods in Enzymology, 322, 480-492 (English) 2000. CODEN: MENZAU. ISSN: 0076-6879. Publisher: Academic Press.
AB A review, with 30 refs., of methods and protocols for investigating effects of a gene of interest on apoptosis. Transient transfection assays are recommended as the first step in investigating the effects of expression of a gene on cell division, growth, or survival esp. when it may be difficult to establish stable cell lines because expression of the gene has neg. effects. The authors list reporter genes which are used to identify transfected cells expressing the gene of interest. Loss of reporter gene expression may indicate cell death or inhibition of gene expression, as long as the transfection efficiency is high. Cell morphol. can distinguish live adherent and dead nonadherent cells and uptake of fluorescent DNA-binding dyes identifies dyeing cells. Nuclear morphol. of

cells stained with acridine orange and ethidium bromide can distinguish normal and apoptotic nuclei from necrotic nuclei. Activation of caspase activity after transfection is another way to measure apoptosis. Finally, DNA-fragmentation, which is assocd. with caspase-mediated cell death or necrosis, can be detected in cells by the TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling) method. (c) 2000 Academic Press.

L11 ANSWER 8 OF 16 CAPLUS COPYRIGHT 2003 ACS on STN
2000:2331 Document No. 132:149376 Caspases: multifunctional proteases. Elkon, Keith B. (Hospital for Special Surgery, Weill Medical College of Cornell University, New York, NY, 10021, USA). Journal of Experimental Medicine, 190(12), 1725-1727 (English) 1999. CODEN: JEMEAV. ISSN: 0022-1007. Publisher: Rockefeller University Press.
AB A review with 22 refs. on functions of caspases with emphasis on role of caspases in apoptosis and cell proliferation. Role of caspases in diseases such as lymphomas, leukemias and disease of immune system is also discussed.

L11 ANSWER 9 OF 16 CAPLUS COPYRIGHT 2003 ACS on STN
2000:28394 Document No. 132:219942 Caspase knockouts: matters of life and death. Zheng, T. S.; Hunot, S.; Kuida, K.; Flavell, R. A. (Section of Immunobiology, Yale University School of Medicine, New Haven, CT, 06510, USA). Cell Death and Differentiation, 6(11), 1043-1053 (English) 1999. CODEN: CDDIEK. ISSN: 1350-9047. Publisher: Stockton Press.
AB A review with 65 refs. Apoptosis, the seemingly counter-intuitive act of physiol. cell suicide, is accomplished by an evolutionarily conserved death program that is centered on the activation of a group of intracellular cysteine proteases known as caspases. It is now clear that both extra- and intra-cellular stimuli induce apoptosis by triggering the activation of these otherwise latent proteases in a process that culminates in caspase-mediated disintegration of cellular contents and their subsequent absorption by neighboring cells. While many elegant in vitro studies have demonstrated the requirement of caspase activities for the execution of most, if not all, apoptosis, the precise contribution of individual caspases in vivo and how they functionally relate to each other remain poorly elucidated. Fortunately, the generation of various caspase deficient mice through gene targeting has provided a unique window of opportunity to definitely examine the physiol. function of these caspases in vivo. As the list of caspase knockouts grows, we considered it was time to review what we have been learned, from these studies about the exact role of individual caspases in mediating apoptotic events. We will also provide our prediction on the direction of future studies in this ever-growing field of caspases.

L11 ANSWER 10 OF 16 CAPLUS COPYRIGHT 2003 ACS on STN
1999:707211 Document No. 132:21339 Poly(ADP-ribosylation) and apoptosis. Scovassi, A. Ivana; Poirier, Guy G. (Istituto di Genetica Biochimica ed Evoluzionistica del C.N.R., Pavia, I-27100, Italy). Molecular and Cellular Biochemistry, 199(1&2), 125-137 (English) 1999. CODEN: MCBIB8. ISSN: 0300-8177. Publisher: Kluwer Academic Publishers.
AB A review with 128 refs. Poly(ADP-ribosylation) is a post-translational modification playing a relevant role in DNA damage recovery, DNA replication and viral integration. Several reports also suggest a modulation of this process during cell death by apoptosis. The aim of this review is to discuss the possible involvement of poly(ADP-ribosylation) during apoptosis, by dealing with general considerations on apoptosis, and further examg. the correlation between NAD consumption and cell death, the regulation of poly(ADP-ribose) metab. in apoptotic cells, the effect of poly(ADP-ribose) polymerase inhibition on cell death occurrence and the use of enzyme cleavage as a marker of apoptosis. Finally, the future prospects of the research in this area will be addressed.

L11 ANSWER 11 OF 16 CAPLUS COPYRIGHT 2003 ACS on STN

1999:318352 Document No. 131:143109 On T-cell receptors, apoptosis, and caspase. Mak, Tak W. (Ontario Cancer Institute, Princess Margaret Hospital, Toronto, ON, M5G 2M9, Can.). Immunologist, 7(1/2), 68-70 (English) 1999. CODEN: INOLEG. ISSN: 1192-5612. Publisher: Hogrefe & Huber Publishers.

AB A review with 32 refs. Apoptosis in different tissues induced by various stimuli occurs via a complex web of pathways with different requirements for a variety of downstream effectors. Understanding these mol. players may help in manipulation of the immune system to control autoimmune diseases and infection.

L11 ANSWER 12 OF 16 CAPLUS COPYRIGHT 2003 ACS on STN

2000:9948 Document No. 132:177037 Death by design: mechanism and control of apoptosis. Song, Z.; Steller, H. (68-430, Dept of Biology and Dept of Brain and Cognitive Sciences, Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, MA, USA). Trends in Genetics, 15(12), M49-M52 (English) 1999. CODEN: TRGEE2. ISSN: 0168-9525. Publisher: Elsevier Science Ltd..

AB A review with 50 refs. Active cellular suicide by apoptosis plays important roles in animal development, tissue homeostasis and a wide variety of diseases, including cancer, AIDS, stroke and many neurodegenerative disorders. A central step in the execution of apoptosis is the activation of an unusual class of cysteine proteases, termed caspases, that are widely expressed as inactive zymogens. Originally, the mechanisms for regulating the caspase-based cell death program seemed to be different in Caenorhabditis elegans, mammals and insects. However, recent results suggest that these apparent differences in the control of cell death reflect our incomplete knowledge, rather than genuine mechanistic differences between different organisms.

L11 ANSWER 13 OF 16 CAPLUS COPYRIGHT 2003 ACS on STN

1999:169142 Document No. 131:3046 Apoptosis: molecular mechanisms. Solary, Eric (Unite INSERM 517, Mort cellulaire et cancer, Groupe Biologie et Therapie des Cancers (JE515), Facultes de Medecine et de Pharmacie, Dijon, 21033, Fr.). Comptes Rendus des Seances de la Societe de Biologie et de Ses Filiales, 192(6), 1065-1076 (French) 1998. CODEN: CRSBAW. ISSN: 0037-9026. Publisher: SGS.

AB A review with 35 refs. Apoptosis is a genetically programed cell death that is required for morphogenesis during embryogenic development and for tissue homeostasis in adult organisms. In most cases, apoptosis involves cytochrome c release from mitochondria. In the cytosol, cytochrome c combines with APAF-1 in the presence of ATP to activate caspase-9 that, in turn, activates effector caspases such as caspase-3. Bcl-2 and related proteins control cytochrome c release from the mitochondria whereas IAP (for Inhibitor of APoptosis) mols. modulate the activity of caspases. Plasma membrane receptors such as Fas (CD95, APO-1), characterized by a so-called "death domain" in their cytoplasmic domain, can activate the caspase cascade through adaptor mols. such as FADD (Fas-Assocd. protein with a Death Domain). Dysregulation of the apoptotic machinery plays a role in the pathogenesis of various diseases, and mols. involved in cell death pathways are potential therapeutic targets in immunol., neurol., cancer, infectious and inflammatory diseases.

L11 ANSWER 14 OF 16 CAPLUS COPYRIGHT 2003 ACS on STN

1998:421639 Document No. 129:52382 Detection of activated caspase-3 by a cleavage site-directed antiserum during naturally occurring cell death. Fujita, Eriko; Miho, Yasuko; Urase, Koko; Momoi, Takashi (Natl. Inst. Neurosci., NCNP, Kodaira, 187-8502, Japan). Fragrance Journal, 26(6), 35-42 (Japanese) 1998. CODEN: FUJAD7. ISSN: 0288-9803. Publisher: Fureguransu Janaru Sha.

AB A review with 30 refs., on (1) role of apoptosis in the maintenance of homeostasis in immune and nervous systems, (2) detection of activated caspase-3 (p20/17 fragment) by a cleavage site-directed antiserum, (3) activation mechanisms of caspase-3 by anti-Fas and TNF, (4) roles of caspase-3 in apoptosis of keratinocytes and thymocytes, (5) mol. mechanism

of apoptosis of dorsal root ganglia neurons, (6) involvement of neurotrophic factors, PI3K, Bcl-2, and p75 NGF receptor in neuronal cell death, and (7) regulation of apoptosis by TGF.beta./BMP family, FGF, and other factors.

L11 ANSWER 15 OF 16 CAPLUS COPYRIGHT 2003 ACS on STN
1997:741450 Document No. 128:85466 Errors of homeostasis and deregulated apoptosis. Rinkenberger, Julie L.; Korsmeyer, Stanley J. (Department Anatomy, University California, San Francisco, CA, 94143-0750, USA). Current Opinion in Genetics & Development, 7(5), 589-596 (English) 1997. CODEN: COGDET. ISSN: 0959-437X. Publisher: Current Biology Ltd..
AB A review with 83 refs. Apoptosis research has accelerated with the discovery of genes within a common cell death pathway and evidence for their inter-relationships. Breakthroughs include insights into the mechanism of action of the Bcl-2 family, caspases and their targets, and death receptor complexes. Deregulation of apoptosis is evident in tumors and viral infection, as well as in autoimmune disease, immunodeficiency, neurodegeneration, and infertility.

L11 ANSWER 16 OF 16 CAPLUS COPYRIGHT 2003 ACS on STN
1997:782040 Document No. 128:124879 Nitric oxide and apoptosis: another paradigm for the double-edged role of nitric oxide. Dimmeler, Stefanie; Zeiher, Andreas M. (Molecular Cardiology Group, Department of Internal Medicine IV, University of Frankfurt, Frankfurt, 60590, Germany). Nitric Oxide, 1(4), 275-281 (English) 1997. CODEN: NIOXF5. ISSN: 1089-8603. Publisher: Academic Press.
AB A review, with .apprx.62 refs. Apoptosis plays an important role in the development of the organism but also under various pathol. conditions. Nitric oxide exhibits contradictory effects in the regulation of apoptosis. Both pro- and antiapoptotic effects have been demonstrated. The proapoptotic effects seem to be linked to pathophysiol. conditions, where high amts. of NO are produced by the inducible nitric oxide synthase. In contrast, the continuous release of endothelial NO inhibits apoptosis and may contribute to the antiatherosclerotic function of NO. The present article summarizes these effects and provides insights into the role of NO in apoptotic signal transduction, with special regard to the Bcl-2 homologous proteins, the protease family of caspases and heat shock proteins.

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L3 ANSWER 1 OF 39 CAPLUS COPYRIGHT 2003 ACS on STN
2003:696406 Document No. 139:225508 Use of tumor up-regulated CARD-contg. antagonist of caspase 9 (TUCAN) cDNA for monitoring cancer prognosis and therapy. Reed, John C. (USA). U.S. Pat. Appl. Publ. US 2003165887 A1 20030904, 65 pp., Cont.-in-part of U.S. Ser. No. 388,221. (English). CODEN: USXXCO. APPLICATION: US 2002-141618 20020507. PRIORITY: US 1999-388221 19990901; US 2001-PV289233 20010507; US 2002-PV356934 20020212.

AB The invention provides methods for detg. a prognosis for survival for a cancer patient. One method involves measuring a level of a TUCAN in a neoplastic cell-contg. sample from the cancer patient, and comparing the level of TUCAN in the sample to a ref. level of TUCAN, wherein a low level of TUCAN in the sample correlates with increased survival of the patient. Another method involves measuring a level of TUCAN in a neoplastic cell-contg. sample from the cancer patient, and classifying the patient as belonging to either a first or second group of patients, wherein the first group of patients having low levels of TUCAN is classified as having an increased likelihood of survival compared to the second group of patients having high levels of TUCAN.

L3 ANSWER 2 OF 39 MEDLINE on STN
2003443801 Document Number: 22866425. PubMed ID: 12791649. Sex-specific alterations in neutrophil apoptosis: the role of estradiol and progesterone. Molloy Eleanor J; O'Neill Amanda J; Grantham Julie J; Sheridan-Pereira Margaret; Fitzpatrick John M; Webb David W; Watson R William G. (Department of Surgery, Mater Misericordiae University Hospital, Dublin, Ireland.) BLOOD, (2003 Oct 1) 102 (7) 2653-9. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Women are conferred with greater immunologic and survival benefits compared to men. Female sex steroids contribute to this sexual dimorphism. Furthermore, during human pregnancy when female sex hormones are elevated, neutrophil apoptosis is delayed. This study examines the specific effects of estradiol and progesterone on neutrophil apoptosis and function in healthy adult men and women. We also examined the contribution of these hormones to the persistence and resolution of an inflammatory response. Spontaneous apoptosis was significantly decreased in women compared with men. Physiologic doses of estradiol and progesterone caused a further delay in spontaneous apoptosis in both men and women but did not diminish Fas **antibody**-induced apoptosis. The delay in apoptosis was mediated at the level of the mitochondria with decreased release of cytochrome c, which may alter caspase cleavage and

activity. There were no associated alterations in neutrophil CD11b, but production of reactive oxygen intermediates (ROIs) in women was increased. Thus, female sex hormones mediate delayed neutrophil apoptosis in both sexes and enhance female intracellular production of ROIs. Modulating hormonal responses may be an effective therapeutic tool in combating inflammatory diseases.

L3 ANSWER 3 OF 39 MEDLINE on STN

2003089462 Document Number: 22489093. PubMed ID: 12601057. Caspase activation in an experimental model of retinal detachment. Zacks David N; Hanninen Virve; Pantcheva Mina; Ezra Eric; Grosskreutz Cynthia; Miller Joan W. (Retina Service, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, Massachusetts, USA.. davzacks@umich.edu) . INVESTIGATIVE OPHTHALMOLOGY AND VISUAL SCIENCE, (2003 Mar) 44 (3) 1262-7. Journal code: 7703701. ISSN: 0146-0404. Pub. country: United States. Language: English.

AB PURPOSE: To test for apoptotic photoreceptor cell death and caspase activation as a function of time after induction of an experimental retinal detachment. METHODS: Retinal detachments were created in Brown Norway rats by injecting 10% hyaluronic acid into the subretinal space using a transvitreous approach. Light microscopy and terminal dUTP-biotin nick end-labeling (TUNEL) was performed at 1, 3, 5, and 7 days after detachment to assess for the morphologic features associated with apoptosis. Western blot analysis of retinal protein extracts was performed using **antibodies** against caspase-3, -7, and -9 and poly-ADP ribose-polymerase (PARP) at 1, 3, and 5 days after detachment. RESULTS: Light microscopic analysis of detached retinas showed the presence of pyknotic nuclei in the outer nuclear layer and disruption of the normal organization of the photoreceptor outer segments. TUNEL-staining was positive in the outer nuclear layer only in the detached portions of the retina. Western blot analysis confirmed the time-dependent activation of caspase-3, -7, and -9 and PARP in the detached retinas. No morphologic stigmata of apoptosis or caspase activation was detected in attached retinas. CONCLUSIONS: The apoptotic photoreceptor cell death in experimental retinal detachments is associated with caspase activation.

L3 ANSWER 4 OF 39 MEDLINE on STN

2003102631 Document Number: 22502557. PubMed ID: 12616497. Perforin-dependent activation-induced cell death acts through caspase 3 but not through caspases 8 or 9. Chen Liane; Woo Minna; Hakem Razqallah; Miller Richard G. (Department of Medical Biophysics, University of Toronto and Ontario Cancer Institute, 610 University Avenue, Toronto, Ontario, Canada M5G 2M9.) EUROPEAN JOURNAL OF IMMUNOLOGY, (2003 Mar) 33 (3) 769-78. Journal code: 1273201. ISSN: 0014-2980. Pub. country: Germany: Germany, Federal Republic of. Language: English.

AB Activation-induced cell death (AICD) is a phenomenon in which activated T cells undergo apoptosis upon restimulation. We are studying a form of AICD that can occur before cells become competent to die by Fas (hence "early" AICD) and which depends on the presence of perforin. Previous studies indicate that it does not occur through granule exocytosis but via some endogenous pathway. We here investigate a possible role for caspases. Caspase 3(-/-) cells were protected, suggesting a role for caspase 3 in early AICD. After recrosslinking, caspase 3 activity could be detected in cell lysates between 3 and 12 h, and CD8(+) T cells became annexin V-positive between 15 and 18 h. Blocking anti-Fas ligand **antibody** failed to inhibit death, and no processing of either caspase 8 or caspase 9 was detected in recrosslinked cells. Furthermore, T cells lacking functional caspase 9 continued to die in early AICD. Thus, perforin-dependent early AICD appears to require activation of caspase 3, but not caspases 8 or 9. As perforin has no intrinsic catalytic abilities, we propose that it releases some endogenous activity that can activate caspase 3.

L3 ANSWER 5 OF 39 MEDLINE on STN

2003126407 Document Number: 22527533. PubMed ID: 12639677. Role of mitochondrial cytochrome c in cocaine-induced apoptosis in rat testes. Li Haikun; Xu Liping; Dunbar Joseph C; Dhabuwala C B. (Department of Urology, Wayne State University School of Medicine, Detroit, Michigan 48201, USA.) UROLOGY, (2003 Mar) 61 (3) 646-50. Journal code: 0366151. ISSN: 1527-9995. Pub. country: United States. Language: English.

AB OBJECTIVES: We have previously demonstrated that cocaine exposure leads to apoptosis in rat testes. To understand further the mechanism of cocaine-induced testicular damage, we studied the effect of cocaine on cytochrome c release from the mitochondria. We also determined the caspase 3, caspase 8, and caspase 9 activities in rat testes after chronic cocaine exposure. METHODS: Thirty-day-old male Sprague-Dawley rats received cocaine hydrochloride or equal volumes of normal saline subcutaneously daily for 90 days. The testes were removed at 15, 30, and 90 days of cocaine or saline administration. Mitochondria and cytosolic fractions from testes were isolated. Western blotting was performed in both fractions using anti-cytochrome c **antibody**. Caspase 3, caspase 8, and caspase 9 activities were determined by fluorometric assay. RESULTS: The expression of cytochrome c protein in the cytosolic fraction was increased on day 15 and persisted for up to 90 days after cocaine injection compared with controls. However, the expression of cytochrome c in testes was decreased in the mitochondria fraction on days 15, 30, and 90 after cocaine injections compared with the corresponding controls. The caspase activity study showed caspase 3 and caspase 9 activities increased in cocaine-treated testes at each point of the study compared with the corresponding controls. However, the caspase 8 activity in cocaine-treated testes did not change significantly at each point of the study compared with the corresponding controls. CONCLUSIONS: Our results suggest that the release of cytochrome c from mitochondria and its subsequent activation of caspase 9 and caspase 3 in testes play a key role in cocaine-induced germ cell apoptosis. Our findings also indicate that cocaine-induced testicular germ cell apoptosis in rats is at least initiated through a mitochondria-associated pathway.

L3 ANSWER 6 OF 39 MEDLINE on STN

2003383954 Document Number: 22801418. PubMed ID: 12920191. Critical role for Akt1 in the modulation of apoptotic phosphatidylserine exposure and microglial activation. Kang Jing-Qiong; Chong Zhao Zhong; Maiese Kenneth. (Department of Neurology, 8C-1 UHC, Wayne State University School of Medicine, 4201 St. Antoine, Detroit, MI 48201, USA.) MOLECULAR PHARMACOLOGY, (2003 Sep) 64 (3) 557-69. Journal code: 0035623. ISSN: 0026-895X. Pub. country: United States. Language: English.

AB Biological targets for neurodegenerative disease that focus on the intrinsic maintenance of cellular integrity and the extrinsic prevention of phagocytic cellular disposal offer the greatest promise for therapeutic intervention. Protein kinase B (Akt1), a serine-threonine kinase closely involved in cell growth and survival, offers a strong potential to address both intrinsic and extrinsic mechanisms of neuronal injury. We demonstrate that overexpression of a constitutively active form of Akt1 (myristoylated Akt1) in differentiated SH-SY5Y neuronal cells provides intrinsic cellular protection against apoptotic genomic DNA destruction and membrane phosphatidylserine (PS) exposure. Transfection of SH-SY5Y cells with a plasmid encoding a kinase-deficient dominant-negative Akt1 eliminates cytoprotection, suggesting that activation of Akt1 is necessary and sufficient to prevent apoptotic destruction. Apoptotic neuronal membrane PS exposure provides a unique pathway for Akt1 to offer extrinsic cellular protection and block microglial activation, because independent cotreatment with an anti-PS receptor neutralizing **antibody** could also prevent microglial proliferation. Akt1 maintains nuclear DNA integrity and membrane PS exposure through the specific inhibition of caspase 3-, 8-, and 9-like activities that were linked to mitochondrial membrane potential and cytochrome c release. Our work elucidates a novel capacity for Akt1 to maintain cellular integrity through a series of cysteine protease pathways and to uniquely regulate microglial activation through the modulation of membrane PS residue externalization.

L3 ANSWER 7 OF 39 CAPLUS COPYRIGHT 2003 ACS on STN
2002:833504 Document No. 137:358061 Conserved sequence of XIAP-binding motif in human caspase-9 and Smac/DIABLO and therapeutic uses for screening modulators of apoptosis. Alnemri, Emad S. (Thomas Jefferson University, USA). U.S. Pat. Appl. Publ. US 2002160975 A1 20021031, 52 pp., Cont.-in-part of U.S. Ser. No. 939,293. (English). CODEN: USXXCO. APPLICATION: US 2002-68569 20020206. PRIORITY: US 2001-PV267966 20010208; US 2001-939293 20010824.

AB The invention provides conserved sequence of XIAP-binding motif in human caspase-9 and Smac/DIABLO. The invention also provides caspase-9-related peptides and polypeptides capable of binding to an Inhibitor of Apoptosis Protein (IAP), as well as caspase-9 mutant that fail to undergo normal processing and fail to bind to an IAP. Nucleic acid mols., including expression vectors, encoding such peptides and polypeptides are also provided. Such peptides and polypeptides, are useful for inducing apoptosis and identifying inhibitors and enhancer of apoptosis.

L3 ANSWER 8 OF 39 MEDLINE on STN
2002417757 Document Number: 22161904. PubMed ID: 12171907. Combretastatin-A4 prodrug induces mitotic catastrophe in chronic lymphocytic leukemia cell line independent of caspase activation and poly(ADP-ribose) polymerase cleavage. Nabha Sanaa M; Mohammad Ramzi M; Dandashi Mahmoud H; Coupaye-Gerard Brigitte; Aboukameel Amro; Pettit George R; Al-Katib Ayad M. (Division of Hematology and Oncology, Department of Internal Medicine, Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, Michigan 48201, USA.) CLINICAL CANCER RESEARCH, (2002 Aug) 8 (8) 2735-41. Journal code: 9502500. ISSN: 1078-0432. Pub. country: United States. Language: English.

AB We have previously reported that combretastatin-A4 prodrug (CA4P), anantitubulin/antiangiogenic agent isolated from the South African willow tree Combretum caffrum, induced cell death primarily through mitotic catastrophe in a panel of human B-lymphoid tumors. In this study, we investigated the molecular aspects of the mitotic catastrophe and whether or not it shares the same pathways of apoptosis. For this we studied the effect of CA4P on selected markers of apoptosis [caspases 9 and 3, poly(ADP-ribose) polymerase (PARP), bcl-2, and bax] and G2-M protein regulators (p53, MDM2, 14-3-3sigma, GADD45, cdc2, cdc25, chk1, wee1, p21, and cyclin B1). The chronic lymphocytic leukemia cell line WSU-CLL was used for this purpose. Western blot analysis showed that 24 h of CA4P (5 nM) exposure induces caspase 9 activation and PARP cleavage. However, the addition of Z-Val-Ala-Asp-fluoromethylketone (a general caspase inhibitor) or Z-Leu-Glu(OMe)-His-Asp(OMe)-CH2F (a caspase 9 inhibitor) before CA4P treatment did not block cell death. No change in bcl-2 or bax protein expression was observed. Exposure of WSU-CLL cells to 4 and 5 nM CA4P was associated with overproduction of total p53 and no dramatic change in MDM2, 14-3-3sigma, GADD45, the cyclin-dependent kinase cdc2, its inhibitory phosphorylation, the cdc2-inhibitory kinase (wee1), chk1, or cdc25 hyperphosphorylation. The overaccumulation of p21 and cyclin B1 protein was obvious at 24 h. Furthermore, CA4P treatment showed an increase in the expression of a marker of mitosis (mitotic protein monoclonal-2 **antibody**) and an overaccumulation of the cyclin B in the nucleus. Our findings suggest that CA4P induces mitotic catastrophe and arrest of WSU-CLL cells mostly in the M phase independent of p53 and independent of chk1 and cdc2 phosphorylation pathways. Apoptosis is a secondary mechanism of death in a small proportion of cells through activation of caspase 9 and PARP cleavage. The two mechanisms of cell death, i.e., mitotic catastrophe and apoptosis, are independent of each other in our model.

L3 ANSWER 9 OF 39 MEDLINE on STN
2003014489 Document Number: 22395753. PubMed ID: 12507932. Boswellic acids trigger apoptosis via a pathway dependent on caspase-8 activation but independent on Fas/Fas ligand interaction in colon cancer HT-29 cells. Liu Jian-Jun; Nilsson Ake; Oredsson Stina; Badmaev Vladimir; Zhao

Wan-Zhou; Duan Rui-Dong. (Cell Biology B, Biomedical Center, B11, Lund University, Sweden.) CARCINOGENESIS, (2002 Dec) 23 (12) 2087-93. Journal code: 8008055. ISSN: 0143-3334. Pub. country: England: United Kingdom. Language: English.

AB Boswellic acids are the effective components of gum resin of Boswellia serrata, which has anti-inflammatory properties. Recent studies on brain tumors and leukemic cells indicate that boswellic acids may have antiproliferative and apoptotic effects with the mechanisms being not studied in detail. We studied their antiproliferative and apoptotic effects on colon cancer cells and the pathway leading to apoptosis. HT-29 cells were treated with beta-boswellic acid (BA), keto-beta-boswellic acid (K-BA) and acetyl-keto-beta-boswellic acid (AK-BA), respectively. Apoptosis was determined by flow cytometry, by cytoplasmic DNA-histone complex and the activity of caspase-3. The cleavage of poly-(ADP-ribose)-polymerase (PARP) and expression of Fas were examined by western blot. Specific caspase inhibitors, polyclonal Fas **antibody**, and antagonistic Fas **antibody** ZB4 were employed to elucidate apoptotic pathways. DNA synthesis and cell viability were examined. Both K-BA and AK-BA increased cytoplasmic DNA-histone complex dose-dependently and increased pre-G(1) peak in flow cytometer analysis, with the effects of AK-BA being stronger than K-BA. BA only increased the formation of DNA-histone complex at a high concentration. K-BA and AK-BA increased caspase-8, caspase-9 and caspase-3 activities accompanied by cleavage of PARP. The effects of AK-BA on formation of cytoplasmic DNA histone and on caspase-3 activation were 3.7- and 3.4-fold, respectively, more effective than those induced by camptothecin. The apoptosis induced by AK-BA was inhibited completely by caspase-3 or caspase-8 inhibitor and partially by caspase-9 inhibitor. ZB4 blocked exogenous Fas ligand-induced apoptosis, but had no effect on AK-BA-induced apoptosis. AK-BA had no significant effect on expression of Fas. Apart from apoptotic effect, these acids also inhibited [(3)H]thymidine incorporation and cell viability to different extent. In conclusion, boswellic acids, particularly AK-BA and K-BA have antiproliferative and apoptotic effects in human HT-29 cells. The apoptotic effect is mediated via a pathway dependent on caspase-8 activation but independent of Fas/FasL interaction.

L3 ANSWER 10 OF 39 MEDLINE on STN

2002079655 Document Number: 21648694. PubMed ID: 11698395. Apicidin, a histone deacetylase inhibitor, induces apoptosis and Fas/Fas ligand expression in human acute promyelocytic leukemia cells. Kwon So Hee; Ahn Seong Hoon; Kim Yong Kee; Bae Gyu-Un; Yoon Jong Woo; Hong Sungyoul; Lee Hoi Young; Lee Yin-Won; Lee Hyang-Woo; Han Jeung-Whan. (Department of Biochemistry and Molecular Biology, College of Pharmacy and Department of Genetic Engineering, College of Life Science and Natural Resources, Sungkyunkwan University, Suwon 440-746, Korea.) JOURNAL OF BIOLOGICAL CHEMISTRY, (2002 Jan 18) 277 (3) 2073-80. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB We previously reported that apicidin arrested human cancer cell growth through selective induction of p21(WAF1/Cip1). In this study, the apoptotic potential of apicidin and its mechanism in HL60 cells was investigated. Treatment of HL60 cells with apicidin caused a decrease in viable cell number in a dose-dependent manner and an increase in DNA fragmentation, nuclear morphological change, and apoptotic body formation, concomitant with progressive accumulation of hyperacetylated histone H4. In addition, apicidin converted the procaspase-3 form to catalytically active effector protease, resulting in subsequent cleavages of poly(ADP-ribose) polymerase and p21(WAF1/Cip1). Incubation of HL60 cells with z-DEVD-fmk, a caspase-3 inhibitor, almost completely abrogated apicidin-induced activation of caspase-3, DNA fragmentation, and cleavages of poly(ADP-ribose) polymerase and p21(WAF1/Cip1). Moreover, these effects were preceded by an increase in translocation of Bax into the mitochondria, resulting in the release of cytochrome c and cleavage of procaspase-9. The addition of cycloheximide greatly inhibited activation of caspase-3 by apicidin by interfering with cleavage of procaspase-3 and

DNA fragmentation, suggesting that apicidin-induced apoptosis was dependent on de novo protein synthesis. Consistent with these results, apicidin transiently increased the expressions of both Fas and Fas ligand. Preincubation with NOK-1 monoclonal **antibody**, which prevents the Fas-Fas ligand interaction and is inhibitory to Fas signaling, interfered with apicidin-induced translocation of Bax, cytochrome c release, cleavage of procaspase-3, and DNA fragmentation. Taken together, the results suggest that apicidin might induce apoptosis through selective induction of Fas/Fas ligand, resulting in the release of cytochrome c from the mitochondria to the cytosol and subsequent activation of caspase-9 and caspase-3.

L3 ANSWER 11 OF 39 MEDLINE on STN

2003010612 Document Number: 22404679. PubMed ID: 12516968. Fas-mediated signaling enhances sensitivity of human soft tissue sarcoma cells to anticancer drugs by activation of p38 kinase. Li WeiWei; Bertino Joseph R. (Program of Molecular Pharmacology and Chemistry, Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center, New York, New York 10021, USA.) Mol Cancer Ther, (2002 Dec) 1 (14) 1343-8. Journal code: 101132535. ISSN: 1535-7163. Pub. country: United States. Language: English.

AB Sensitivity of human soft tissue sarcoma (STS) cells to methotrexate, doxorubicin, and paclitaxel was examined after cells were pretreated with CH-11, an agonistic anti-Fas **antibody**. A subtoxic dose (6 ng/ml) of CH-11 sensitized STS cells but not normal fibroblast cells to these anticancer drugs. CH-11 increased cytochrome c release and consequent activation of caspase-9, independent of caspase-8 and increased p38 activation. Addition of SB203580, a specific inhibitor of p38, resulted in a decrease in activation of this kinase and abrogation of enhanced chemosensitivity (doxorubicin and paclitaxel) by CH-11. These results demonstrate that stimulation of the Fas pathway by a subtoxic dose of a Fas agonist can selectively enhance sensitivity of STS cells to certain chemotherapeutic agents through activation of p38.

L3 ANSWER 12 OF 39 MEDLINE on STN

2002719934 Document Number: 22369815. PubMed ID: 12481428. X-linked inhibitor of apoptosis (XIAP) blocks Apo2 ligand/tumor necrosis factor-related apoptosis-inducing ligand-mediated apoptosis of prostate cancer cells in the presence of mitochondrial activation: sensitization by overexpression of second mitochondria-derived activator of caspase/direct IAP-binding protein with low pl (Smac/DIABLO). Ng Chuen-Pei; Bonavida Benjamin. (Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles School of Medicine, Jonsson Comprehensive Cancer Center, University of California, Los Angeles, California 90095, USA.) Mol Cancer Ther, (2002 Oct) 1 (12) 1051-8. Journal code: 101132535. ISSN: 1535-7163. Pub. country: United States. Language: English.

AB The resistance to Apo2 ligand (Apo2L)/tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis could be overcome by treatment with subtoxic concentrations of actinomycin D (Act D) in prostate tumor cells. Furthermore, the sensitization to Apo2L/TRAIL-mediated apoptosis by Act D positively correlated with selective down-regulation of X-linked inhibitor of apoptosis (XIAP). In this study, we examined whether second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low pl (Smac/DIABLO), a known inhibitor of apoptosis (IAP)-neutralizing protein, sensitizes resistant prostate tumor cells to Apo2L/TRAIL-mediated apoptosis. The prostate tumor cell line CL-1 was treated with Apo2L/TRAIL, Act D, or a combination of the two. The apoptosis-mediated signaling pathway was examined by Western blotting and flow cytometry. Furthermore, CL-1 cells transfected with the anti-IAP inhibitor Smac/DIABLO were examined for sensitivity to Apo2L/TRAIL. Whereas Apo2L/TRAIL induced the release of cytochrome c and endogenous Smac/DIABLO in the CL-1 tumor cells, the cytosolic levels of both molecules were not sufficient to induce apoptosis. Transient transfectants with a

Smac/DIABLO cDNA encoding a neutralizing inhibitor of IAPs were sensitized to Apo2L/TRAIL-mediated apoptosis. The sensitization to Apo2L/TRAIL by Smac/DIABLO overexpression was a result of synergistic activation of caspases-3, -9, and -8. Treatment of the Smac/DIABLO transient transfectant with Apo2L/TRAIL enhanced the release of Smac/DIABLO from mitochondria and led to reduction of IAP family proteins (XIAP, c-IAP1, and c-IAP2). These results show that Smac/DIABLO can sensitize CL-1 tumor cells to Apo2L/TRAIL-mediated apoptosis. Thus, up-regulation of Smac/DIABLO and sensitization to Apo2L/TRAIL-mediated apoptosis are of potential clinical application in the immunotherapy of drug-/Apo2L/TRAIL-resistant tumors.

L3 ANSWER 13 OF 39 MEDLINE on STN

2002080583 Document Number: 21665829. PubMed ID: 11807010. The mechanism of tumor cell clearance by rituximab in vivo in patients with B-cell chronic lymphocytic leukemia: evidence of caspase activation and apoptosis induction. Byrd John C; Kitada Shinichi; Flinn Ian W; Aron Jennifer L; Pearson Michael; Lucas David; Reed John C. (Division of Hematology-Oncology, The Ohio State University, B302 Starling Loving Hall, 320 W 10th Ave, Columbus, OH 43210, USA.. byrd-3@medctr.ohsu.edu) . BLOOD, (2002 Feb 1) 99 (3) 1038-43. Journal code: 7603509. ISSN: 0006-4971. Pub. country: United States. Language: English.

AB Rituximab is a chimeric monoclonal **antibody** directed at CD20 with significant activity in non-Hodgkin lymphoma (NHL) and chronic lymphocytic leukemia (CLL). A variety of pathways of tumor cytotoxicity different from cytotoxic chemotherapy have been proposed for this therapeutic **antibody** including **antibody**-dependent cellular cytotoxicity and complement-mediated cell lysis. This report describes that a proportion of patients with CLL receiving rituximab treatment have in vivo activation of caspase-9, caspase-3, and poly(ADP-ribose) polymerase (PARP) cleavage in blood leukemia cells immediately following infusion of rituximab. This suggests that apoptosis using a pathway similar to fludarabine and other chemotherapeutic agents is intricately involved in the blood elimination of tumor cells after rituximab treatment. Patients having caspase-3 activation and PARP cleavage in vivo had a significantly lower blood leukemia cell count after treatment as compared to those without caspase activation. Significant down-modulation of the antiapoptotic proteins XIAP and Mcl-1 was also noted, possibly explaining in part how rituximab sensitizes CLL cells to the cytotoxic effect of chemotherapy in vivo. These findings suggest that the therapeutic benefit of **antibody**-based therapy in vivo for patients with CLL depends in part on induction of apoptosis and provides another area of focus for studying mechanisms of **antibody** -resistance in neoplastic cells.

L3 ANSWER 14 OF 39 MEDLINE on STN

2002058345 Document Number: 21630048. PubMed ID: 11756562. Suppression of Akt signaling induces Fas ligand expression: involvement of caspase and Jun kinase activation in Akt-mediated Fas ligand regulation. Suhara Toshimitsu; Kim Hyo-Soo; Kirshenbaum Lorrie A; Walsh Kenneth. (Division of Cardiovascular Research, St. Elizabeth's Medical Center of Boston, Massachusetts 02135, USA.) MOLECULAR AND CELLULAR BIOLOGY, (2002 Jan) 22 (2) 680-91. Journal code: 8109087. ISSN: 0270-7306. Pub. country: United States. Language: English.

AB Fas and Fas ligand (FasL) expression has been detected in chronic vascular lesions, and Fas-mediated apoptosis of vascular smooth muscle cells (VSMC) may influence the integrity of the atherosclerotic plaque. Here we report that FasL is not expressed by normal VSMC, but its expression is upregulated by stresses that induce apoptosis, including serum deprivation, exposure to the phosphatidylinositol 3-kinase (PI 3-kinase) inhibitor wortmannin, and ablation of Akt signaling. Conversely, constitutive activation of Akt signaling diminished FasL expression in VSMC cultures exposed to low-mitogen media or wortmannin. Under conditions of suppressed PI 3-kinase/Akt signaling, VSMC apoptosis was partially inhibited by treatment with neutralizing **antibody**

against FasL. Suppression of Akt signaling increased the activity of c-Jun N-terminal kinase, and transduction of dominant-negative c-Jun inhibited FasL induction under these conditions. Diminished Akt signaling promoted the cleavage of caspase 3, and both caspase 3 cleavage and FasL induction were inhibited by transduction of dominant-negative caspase 9 or the caspase 8 inhibitor CrmA. Similarly, induction of FasL by the Akt-regulated forkhead transcription factor FKHRL1 was dependent upon caspase and c-Jun activation. Taken together, these results indicate that the sequential activation of caspase 3 and c-Jun participates in the induction of FasL under conditions of suppressed Akt signaling or FKHRL1 activation and that FasL participates in a positive-feedback loop to promote cell death under conditions of cellular stress.

L3 ANSWER 15 OF 39 MEDLINE on STN

2002670958 Document Number: 22318732. PubMed ID: 12432255. Downregulation of c-FLIP sensitizes DU145 prostate cancer cells to Fas-mediated apoptosis. Hyer Marc L; Sudarshan Sunil; Kim Youngsoo; Reed John C; Dong Jian-yun; Schwartz David A; Norris James S. (Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, South Carolina 29425, USA.) Cancer Biol Ther, (2002 Jul-Aug) 1 (4) 401-6. Journal code: 101137842. ISSN: 1538-4047. Pub. country: United States. Language: English.

AB Although DU145 prostate cancer cells are resistant to exogenously applied Fas agonist CH-11 (anti-Fas monoclonal **antibody**), Fas-resistance can be overcome using a FasL expressing adenovirus (AdGFPFasL(TET)) [Hyer et al., Molecular Therapy, 2000; 2:348-58 (ref.12)]. The purpose of this study was to try to understand why DU145 cells are resistant to CH-11 and determine the signaling pathway utilized by AdGFPFasL(TET) to induce apoptosis in these Fas-resistant cells. Using immunoblot analysis, we show that AdGFPFasL(TET) is capable of initiating the classic Fas-mediated apoptotic pathway in DU145 cells, which includes activation of caspases-8, -3, -7, and -9, BID cleavage, cytochrome c release from mitochondria, and PARP cleavage. In contrast, CH-11 binds to Fas, but is unable to transmit the death signal beyond the plasma membrane suggesting a block at the DISC (death inducing signaling complex). The anti-apoptotic protein c-FLIP (cellular Flice-like inhibitory protein), which has been shown to inhibit Fas-mediated apoptosis at the DISC, was down-regulated following AdGFPFasL(TET) treatment prompting us to investigate its role in inhibiting CH-11-induced cell death. Using c-FLIP anti-sense oligonucleotides to down-regulate c-FLIP we sensitized DU145 cells to CH-11-induced apoptosis. These data suggest that c-FLIP may play a critical role in regulating Fas-mediated apoptosis in prostate cancer cells and that modulation of c-FLIP may enhance Fas signaling based therapies.

L3 ANSWER 16 OF 39 MEDLINE on STN

2002411066 Document Number: 22155376. PubMed ID: 12164932. Ganglioside loss promotes survival primarily by activating integrin-linked kinase/Akt without phosphoinositide 3-OH kinase signaling. Sun Ping; Wang Xiao-Qi; Lopatka Keith; Bangash Suleman; Paller Amy S. (Department of Pediatrics, Children's Memorial Institute for Education and Research, North-western University Medical School, 2300 Children's Plaza, Chicago, IL 60614, U.S.A.) JOURNAL OF INVESTIGATIVE DERMATOLOGY, (2002 Jul) 119 (1) 107-17. Journal code: 0426720. ISSN: 0022-202X. Pub. country: United States. Language: English.

AB Keratinocyte gangliosides influence cellular functions, including proliferation, adhesion, migration, and differentiation. The effects of endogenous depletion of membrane gangliosides by gene transfection of a human ganglioside-specific sialidase on cell survival were investigated. Ganglioside depletion promotes survival of the human keratinocyte-derived SCC12 cell line through upregulated phosphorylation of beta1 integrin, and increased phosphorylation and activity of integrin-linked kinase, protein kinase B/Akt, and Bad, with resultant inhibition of caspase-9 activation. Ganglioside deficiency also increases expression of cyclins D1 and E, promoting cell cycle progression from G1 phase to S phase. Inhibition of

either protein kinase B/Akt or integrin-linked kinase activity renders the ganglioside-deficient cells susceptible to triggers of apoptosis. Both serine-473 and threonine-308 sites of protein kinase B/Akt show increased phosphorylation in ganglioside-deficient cells, but the cell survival correlates with increased phosphorylation of the serine-473 site of Akt, not with increased phosphorylation of the threonine-308 site. Consistently, blockade of ganglioside GT1b function activates integrin-linked kinase and only the serine-473 site of protein kinase B/Akt. In contrast, **antibody**-induced blockade of GM3 function increases only threonine-308 phosphorylation of ganglioside-deficient cells. Whereas blockade of phosphoinositide 3-OH kinase function suppresses threonine-308 phosphorylation, it neither inhibits serine-473 phosphorylation nor triggers apoptosis. These data suggest that ganglioside depletion modulates cell survival primarily through protein kinase B/Akt stimulation by a pathway that does not require phosphoinositide 3-OH kinase and epidermal growth factor receptor signaling.

L3 ANSWER 17 OF 39 MEDLINE on STN

2002128342 Document Number: 21852550. PubMed ID: 11865194. Activation of caspase-8 is critical for sensitivity to cytotoxic anti-Fas **antibody**-induced apoptosis in human ovarian cancer cells. Hayakawa A; Wu J; Kawamoto Y; Zhou Y W; Tanuma S; Nakashima I; Suzuki H. (Department of Equipment Center for Research and Education, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan.) APOPTOSIS, (2002 Apr) 7 (2) 107-13. Journal code: 9712129. ISSN: 1360-8185. Pub. country: United States. Language: English.

AB Two ovarian cancer cell lines named NOS4 and SKOV-3 have been shown to have different sensitivities to a cytotoxic anti-Fas **antibody**, CH-11. Although both cell lines express Fas molecules on the cell surfaces at the same intensities, apoptosis is induced by CH-11 in NOS4 cells but not in SKOV-3 cells. In this study, the different apoptosis-sensitivities of these cells were assessed. Both cell lines express almost the same levels of FADD, RIP, c-FLIP, FAP-1, Bax, Bcl-2 and Bcl-XL. Evidence of caspase-8, caspase-9 and caspase-3 activation and of cleavage of PARP and Bid was obtained in NOS4 cells but not in SKOV-3 cells. When triggered by FasL protein, DNA fragmentation and caspase-8 activation were observed in SKOV-3 cells, though they were not as clear as in NOS4 cells. All the anti-Fas **antibody**-mediated signals for apoptosis induction in NOS4 cells were completely blocked by a caspase-8-specific inhibitor, Z-IETD-FMK. These results indicate that the different sensitivities to the anti-Fas **antibody** are solely dependent on the activation of caspase-8, which could be influenced by yet unknown qualitative or quantitative abnormalities in molecules involved in DISC formation.

L3 ANSWER 18 OF 39 MEDLINE on STN

2002001289 Document Number: 21621066. PubMed ID: 11751162. Cytokine regulation of human intestinal primary epithelial cell susceptibility to Fas-mediated apoptosis. Martin Carla A; Panja Asit. (Gastrointestinal Research Laboratory, Division of Gastroenterology Hepatology and Nutrition, Department of Medicine, Winthrop-University Hospital, Mineola, New York 11501, USA.) AMERICAN JOURNAL OF PHYSIOLOGY. GASTROINTESTINAL AND LIVER PHYSIOLOGY, (2002 Jan) 282 (1) G92-G104. Journal code: 100901227. ISSN: 0193-1857. Pub. country: United States. Language: English.

AB The regulatory mechanisms of nontransformed intestinal epithelial cell apoptosis have not been thoroughly investigated. We determined the susceptibility and mechanism of Fas-mediated apoptosis in nontransformed human intestinal epithelial cells (HIPEC) in the presence and absence of inflammatory cytokines. Despite ample expression of Fas, HIPEC were relatively insensitive to Fas-mediated apoptosis in that agonist anti-Fas **antibody** (CH11) induced a <25% increase in HIPEC apoptosis. Pretreatment of HIPEC with interferon (IFN)-gamma, but not tumor necrosis factor-alpha or granulocyte-macrophage colony-stimulating factor,

significantly increased CH11-induced apoptosis of these cells without increasing Fas expression. Increased apoptosis correlated with increased caspase 3 activation but not expression of procaspase 3. Also, there was a significant delay in the onset of Fas-mediated apoptosis in HIPEC, which correlated with the generation of an activated caspase 3 p22/20 subunit. HIPEC required both initiator caspases 8 and 9 activity but expressed significantly less of the zymogen form of these caspases than did control cells. IFN-gamma-mediated sensitization of HIPEC occurred upstream of caspase 9 activation and correlated with a small increase in procaspase 8 expression (<1-fold increase) and a significant increase in expression of an intermediate form (p35) of caspase 4 (3.3-fold increase).

L3 ANSWER 19 OF 39 MEDLINE on STN

2002128351 Document Number: 21852548. PubMed ID: 11865192. Thrombocytopenia in an animal model of malaria is associated with an increased caspase-mediated death of thrombocytes. Piguet P F; Kan C D; Vesin C. (Department of Pathology, University of Geneva, CH 1211, Switzerland.. pierre.piguet@medecine.unige.ch) . APOPTOSIS, (2002 Apr) 7 (2) 91-8. Journal code: 9712129. ISSN: 1360-8185. Pub. country: United States. Language: English.

AB Infection of mice with Plasmodium Berghei Anka (PbA) leads to a thrombocytopenia, due to a reduced platelet life span, eventually associated with a syndrome of severe or cerebral malaria (CM). Thrombocytopenia was associated with an increase in the number of microparticles (mcp) in plasma. More than >60% of these mcp were of platelet origin, as seen by staining with an anti-platelet **antibody**. The thrombocytopenia and the amount of mcp were decreased in mice treated with anti CD40L mAb, suggesting that CD40L is the main effector of the thrombocytopenia. Caspase-1, -3, -6, -8, -9 were activated in platelets from infected mice, as seen by the binding of labeled probes or the amount of pro-caspase-3. Treatment of infected mice with the caspases inhibitor ZVAD-fmk decreased the number of mcp and the thrombocytopenia, showing that platelet caspases are responsible for platelet fragmentation. In addition, the caspase inhibitor also caused a decrease in the mortality associated with CM, indicating a critical role of caspases in the expression of CM.

L3 ANSWER 20 OF 39 MEDLINE on STN

2002076720 Document Number: 21661698. PubMed ID: 11803376. Pre-processed caspase-9 contained in mitochondria participates in apoptosis. Costantini P; Bruey J-M; Castedo M; Metivier D; Loeffler M; Susin S A; Ravagnan L; Zamzami N; Garrido C; Kroemer Guido. (Centre National de la Recherche Scientifique, UMR1599, Institut Gustave Roussy, 39 rue Camille-Desmoulins, F-94805 Villejuif, France.) CELL DEATH AND DIFFERENTIATION, (2002 Jan) 9 (1) 82-8. Journal code: 9437445. ISSN: 1350-9047. Pub. country: England: United Kingdom. Language: English.

AB As shown here, mitochondria purified from different organs (liver, brain, kidney, spleen and heart) contain both pro-caspase-9 and the processed, mature form of caspase-9. Purified liver mitochondria release mature caspase-9 upon induction of permeability transition in vitro. This is accompanied by a discrete increase in the enzymatic cleavage of pro-caspase-9 substrates. We found that SHEP neuroblastoma cells constitutively contain pre-processed caspase-9 in their mitochondria, using a combination of subcellular fractionation and immunofluorescence with an **antibody** specific for the processed caspase. This is a cell type-specific phenomenon since HeLa cells mitochondria mainly contain pro-caspase-9 and comparatively little processed caspase-9. Upon introduction of apoptosis, mitochondrial pro-caspase-9 translocates to the cytosol and to the nucleus. This phenomenon is inhibited by transfection with Bcl-2. In synthesis, we report the unexpected finding that mitochondria can contain a pre-processed caspase isoform in non-apoptotic cells. Bcl-2-mediated regulation of mitochondrial membrane permeabilization may contribute to apoptosis control by preventing mitochondrial, pre-processed caspase-9 from interacting with its cytosolic activators.

L3 ANSWER 21 OF 39 MEDLINE on STN

2001673465 Document Number: 21576187. PubMed ID: 11571294. Tumor necrosis factor-alpha induces Bax-Bak interaction and apoptosis, which is inhibited by adenovirus E1B 19K. Sundararajan R; Cuconati A; Nelson D; White E. (Rutgers University, Piscataway, New Jersey 08854, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Nov 30) 276 (48) 45120-7. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Tumor necrosis factor (TNF)-alpha-mediated death signaling induces oligomerization of proapoptotic Bcl-2 family member Bax into a high molecular mass protein complex in mitochondrial membranes. Bax complex formation is associated with the release of cytochrome c, which propagates death signaling by acting as a cofactor for caspase-9 activation. The adenovirus Bcl-2 homologue E1B 19K blocks TNF-alpha-mediated apoptosis by preventing cytochrome c release, caspase-9 activation, and apoptosis of virus-infected cells. TNF-alpha induces E1B 19K-Bax interaction and inhibits Bax oligomerization. Oligomerized Bax may form a pore to release mitochondrial proteins, analogous to the homologous pore-forming domains of bacterial toxins. E1B 19K can also bind to proapoptotic Bak, but the functional significance is not known. TNF-alpha signaling induced Bak-Bax interaction and both Bak and Bax oligomerization. E1B 19K was constitutively in a complex with Bak, and blocked the Bak-Bax interaction and oligomerization of both. The TNF-alpha-mediated cytochrome c and Smac/DIABLO release from mitochondria was inhibited by E1B 19K expression in adenovirus-infected cells. Since either Bax or Bak is essential for death signaling by TNF-alpha, the interaction between E1B 19K and both Bak and Bax may be required to inhibit their cooperative or independent oligomerization to release proteins from mitochondria which promote caspase activation and cell death.

L3 ANSWER 22 OF 39 MEDLINE on STN

2001342984 Document Number: 21299121. PubMed ID: 11406544. Resveratrol induces extensive apoptosis by depolarizing mitochondrial membranes and activating caspase-9 in acute lymphoblastic leukemia cells. Dorrie J; Gerauer H; Wachter Y; Zunino S J. (The Chair of Genetics, Friedrich-Alexander University of Erlangen-Nurnberg, D91058 Erlangen, Germany.) CANCER RESEARCH, (2001 Jun 15) 61 (12) 4731-9. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB Resveratrol, a plant antibiotic, has been found to have anticancer activity and was recently reported to induce apoptosis in the myeloid leukemia line HL60 by the CD95-CD95 ligand pathway. However, many acute lymphoblastic leukemias (ALLs), particularly of B-lineage, are resistant to CD95-mediated apoptosis. Using leukemia lines derived from patients with pro-B t(4;11), pre-B, and T-cell ALL, we show in this report that resveratrol induces extensive apoptotic cell death not only in CD95-sensitive leukemia lines, but also in B-lineage leukemic cells that are resistant to CD95-signaling. Multiple dose treatments of the leukemic cells with 50 microM resveratrol resulted in >/=80% cell death with no statistically significant cytotoxicity against normal peripheral blood mononuclear cells under identical conditions. Resveratrol treatment did not increase CD95 expression or trigger sensitivity to CD95-mediated apoptosis in the ALL lines. Inhibition of CD95-signaling with a CD95-specific antagonistic **antibody** indicated that CD95-CD95 ligand interactions were not involved in initiating resveratrol-induced apoptosis. However, in each ALL line, resveratrol induced progressive loss of mitochondrial membrane potential as measured by the dual emission pattern of the mitochondria-selective dye JC-1. The broad spectrum caspase inhibitor benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone failed to block the depolarization of mitochondrial membranes induced by resveratrol, further indicating that resveratrol action was independent of upstream caspase-8 activation via receptor ligation. However, increases in caspase-9 activity ranged from 4- to 9-fold in the eight cell lines after treatment with resveratrol. Taken together, these results point to a general mechanism of apoptosis induction by resveratrol in ALL cells that involves a mitochondria/caspase-9-specific pathway for the activation

of the caspase cascade and is independent of CD95-signaling.

L3 ANSWER 23 OF 39 MEDLINE on STN
2001347249 Document Number: 21303209. PubMed ID: 11410525. Preferential induction of apoptosis by interferon (IFN)-beta compared with IFN-alpha2: correlation with TRAIL/Apo2L induction in melanoma cell lines. Chawla-Sarkar M; Leaman D W; Borden E C. (Center for Drug Discovery and Development, Taussig Cancer Center and Lerner Research Institute, Cleveland Clinic Foundation, Cleveland, Ohio 44195, USA.) CLINICAL CANCER RESEARCH, (2001 Jun) 7 (6) 1821-31. Journal code: 9502500. ISSN: 1078-0432. Pub. country: United States. Language: English.
AB On the basis of in vitro inhibition of tumor cell growth, IFNs have been generally considered to be antiproliferative proteins. To probe further the potential mechanisms of the antitumor effects of IFNs, we have assessed apoptosis in response to IFN-alpha2 and IFN-beta in cell lines of varied histologies, with a focus on melanomas. Many of the cell lines tested underwent apoptosis in response to IFN-beta, as assessed both by Annexin V and terminal deoxynucleotidyl transferase-mediated nick end labeling staining. In general, IFN-beta had greater growth inhibitory and proapoptotic effects than IFN-alpha2 on all cell lines. The melanoma cell line WM9, sensitive to growth inhibition by IFNs, had a greater degree of apoptosis than A375 melanoma cells, which were largely resistant to antigrowth effects of IFNs. IFN-beta-induced apoptosis was dependent on activation of the caspase cascade with cleavage of caspases 3, 8, and 9 and of the caspase 3 substrate, poly(ADP-ribose) polymerase. Caspase inhibitors benzyloxycarbonyl-Val-Ala-Asp-fluoromethyl keton or benzyloxycarbonyl-Asp-Glu-Val-Asp-fluoromethyl keton, inhibited IFN-beta-induced apoptosis. Other changes associated with apoptosis, including the movement of cytochrome c from mitochondria to cytoplasm and DNA fragmentation, were also identified in response to IFN-beta. Apo2L ligand [tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)] was one of the early genes induced by IFN-beta in apoptosis-sensitive WM9 cells. Other sensitive melanoma cell lines had a similar IFN-beta-specific induction of TRAIL. Neutralizing **antibody** to TRAIL inhibited IFN-beta-induced apoptosis in WM9 cells. In resistant A375 cells, IFN-beta did not induce TRAIL/Apo2L expression. Thus, induction of TRAIL by IFNs in some tumor types may initiate the apoptotic cascade. This study offers another mechanism for the antitumor effects of IFNs.

L3 ANSWER 24 OF 39 MEDLINE on STN
2001416424 Document Number: 21358268. PubMed ID: 11465715. Regulation of CD95 (Fas/APO-1)-induced apoptosis in human chondrocytes. Kuhn K; Lotz M. (The Scripps Research Institute, La Jolla, California 92037, USA.) ARTHRITIS AND RHEUMATISM, (2001 Jul) 44 (7) 1644-53. Journal code: 0370605. ISSN: 0004-3591. Pub. country: United States. Language: English.
AB OBJECTIVE: To examine the role of nuclear factor kappaB (NF-kappaB) and caspases 3, 8, and 9 in CD95-mediated apoptosis of normal chondrocytes. METHODS: First-passage chondrocytes from normal human knee cartilage were stimulated with CD95 **antibody**, and cell death was determined by annexin V binding and by an enzyme-linked immunosorbent assay. Activation of caspases 3, 8, and 9 was measured by Western blotting, and their role in death signaling was evaluated using caspase-specific small peptide inhibitors. The influence of NF-kappaB was determined by electrophoretic mobility shift assay (EMSA) and proteasome inhibition-dependent blocking of the degradation of inhibitor of NF-kappaB. RESULTS: Low levels of NF-kappaB activity were detected by EMSA in unstimulated chondrocytes. NF-kappaB activity was increased in response to agonistic CD95 **antibody**. CD95 **antibody**-induced apoptosis was potentiated by the proteasome inhibitors MG-132 and PS1, and this was associated with a reduced nuclear translocation of NF-kappaB. Proteasome inhibitors also caused the induction of DNA fragmentation by tumor necrosis factor alpha. Procaspase 3 processing was enhanced by the proteasome inhibitor MG-132. Procaspase 8 was undetectable by immunoblotting in whole cell lysates of chondrocytes, but caspase 8

messenger RNA was detected by reverse transcription-polymerase chain reaction. Furthermore, apoptosis induced by CD95 stimulation and proteasome inhibitors was blocked by the caspase 8-specific inhibitor Ac-IETD-CHO. Processing of procaspase 9 was not observed, and inhibition of CD95-dependent cell death by the caspase 9 inhibitor Ac-LEHD-CHO was not significant. CONCLUSION: These results suggest that CD95-dependent cell death is enhanced by NF-kappaB inhibition at and/or downstream of caspase 8 activation and that caspase 9 activation is not involved in CD95-mediated apoptosis in chondrocytes.

L3 ANSWER 25 OF 39 MEDLINE on STN

2001226078 Document Number: 21102142. PubMed ID: 11171371. Segregation of nucleolar components coincides with caspase-3 activation in cisplatin-treated HeLa cells. Horky M; Wurzer G; Kotala V; Anton M; Vojtesek B; Vacha J; Wesierska-Gadek J. (Department of Pathological Physiology, Faculty of Medicine, Masaryk University, Komenskeho namesti 2, 662 43 Brno, Czech Republic.. mhorky@med.muni.cz) . JOURNAL OF CELL SCIENCE, (2001 Feb) 114 (Pt 4) 663-70. Journal code: 0052457. ISSN: 0021-9533. Pub. country: England: United Kingdom. Language: English.

AB We studied morphological changes of the nucleoli in HeLa cells treated with cisplatin and compared them with induction of markers of programmed cell death and TUNEL staining. We used different light microscopic nucleolar staining methods allowing us to visualize not only nucleolar proteins but also nucleolar RNA. Our results show predominantly compact, centrally localized nucleoli in intact control HeLa cells. In cisplatin-treated HeLa cells, we found an early onset of nucleolar segregation of proteins detected by argyrophilic nucleolar organizer regions and anti-nucleolar monoclonal **antibody** as well as an increased immunoreactivity for activated caspase-3 after 6 hours. Staining with Toluidine Blue and Methyl-green Pyronine revealed segregated nucleoli 12 hours after the treatment with cisplatin. TUNEL positivity in cisplatin-treated HeLa cells was accompanied by the aggregation of the argyrophilic proteins in the central portion of nucleus, disappearance of nucleolar RNA and shrinkage of the nucleus after 24 hours. Monitoring of the biochemical changes by immunoblotting revealed that activation of distinct caspases and degradation of their downstream protein substrates is executed in two phases. During an early apoptotic stage beginning 4.5 hours post treatment an activation of caspase-9 and caspase-3 was observed. This was accompanied by proteolytic cleavage of poly(ADP-ribose) polymerase-1 (PARP-1). The caspase-9 activation seems to be mediated by recruitment by the activating factor Apaf-1 because the increased accumulation of Apaf-1 and cytochrome C in cytosol preceded the generation of mature caspase-9 form. A second phase of apoptosis occurring between 10 and 15 hours post treatment was characterized by degradation of other nucleolar and nuclear proteins such as nuclear lamins, topoisomerase I and B23. In conclusion, remarkable segregation of nucleolar argyrophilic proteins, nucleolar RNA and a simultaneous activation of the cascade of caspases markedly preceded the TUNEL positivity in cisplatin-treated HeLa cells thereby substantiating the hypothesis that the nucleolus is a preferred target for caspase-3-dependent proteolysis in cisplatin-treated HeLa cells.

L3 ANSWER 26 OF 39 MEDLINE on STN

2001675616 Document Number: 21562690. PubMed ID: 11696559. alpha-Toxin is a mediator of Staphylococcus aureus-induced cell death and activates caspases via the intrinsic death pathway independently of death receptor signaling. Bantel H; Sinha B; Domschke W; Peters G; Schulze-Osthoff K; Janicke R U. (Department of Immunology and Cell Biology, University of Munster, 48149 Munster, Germany.) JOURNAL OF CELL BIOLOGY, (2001 Nov 12) 155 (4) 637-48. Journal code: 0375356. ISSN: 0021-9525. Pub. country: United States. Language: English.

AB Infections with Staphylococcus aureus, a common inducer of septic and toxic shock, often result in tissue damage and death of various cell types. Although S. aureus was suggested to induce apoptosis, the underlying signal transduction pathways remained elusive. We show that

caspase activation and DNA fragmentation were induced not only when Jurkat T cells were infected with intact bacteria, but also after treatment with supernatants of various S. aureus strains. We also demonstrate that S. aureus-induced cell death and caspase activation were mediated by alpha-toxin, a major cytotoxin of S. aureus, since both events were abrogated by two different anti-alpha-toxin **antibodies** and could not be induced with supernatants of an alpha-toxin-deficient S. aureus strain. Furthermore, alpha-toxin-induced caspase activation in CD95-resistant Jurkat sublines lacking CD95, Fas-activated death domain, or caspase-8 but not in cells stably expressing the antiapoptotic protein Bcl-2. Together with our finding that alpha-toxin induces cytochrome c release in intact cells and, interestingly, also from isolated mitochondria in a Bcl-2-controlled manner, our results demonstrate that S. aureus alpha-toxin triggers caspase activation via the intrinsic death pathway independently of death receptors. Hence, our findings clearly define a signaling pathway used in S. aureus-induced cytotoxicity and may provide a molecular rationale for future therapeutic interventions in bacterial infections.

L3 ANSWER 27 OF 39 MEDLINE on STN

2001360169 Document Number: 21315350. PubMed ID: 11423913. Inhibition of phosphatidylinositol-3 kinase/Akt or mitogen-activated protein kinase signaling sensitizes endothelial cells to TNF-alpha cytotoxicity. Zhang L; Himi T; Morita I; Murota S. (Department of Cellular Physiological Chemistry, Graduate School, Tokyo Medical and Dental University 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8549, Japan.) CELL DEATH AND DIFFERENTIATION, (2001 May) 8 (5) 528-36. Journal code: 9437445. ISSN: 1350-9047. Pub. country: England: United Kingdom. Language: English.

AB Bovine carotid artery endothelial (BAE) cells are resistant to tumor necrosis factor-alpha (TNF), like most other cells. We examined if mitogen-activated protein (MAP) kinase and phosphatidylinositol-3 (PI3) kinase/Akt pathways are involved in this effect. In BAE cells, TNF activates MAP kinase in a MAP kinase kinase 1 (MEK1) manner and Akt in PI3-kinase-dependent manner. Pretreatment with either the MEK1 inhibitor U0126 or PI3-kinase inhibitor LY294002 sensitized BAE cells to TNF-induced apoptosis. Neither U0126 nor LY294002 pretreatment affected TNF-induced activation of NF-kappaB, suggesting that the MAP kinase or PI3-kinase/Akt-mediated anti-apoptotic effect induced by TNF was not relevant to NF-kappaB activation. Both MAP kinase and PI3-kinase/Akt -mediated signaling could prevent cytochrome c release and mitochondrial transmembrane potential (Deltapsi) decrease. PI3-kinase/Akt signaling attenuated caspase-8 activity, whereas MAP kinase signaling impaired caspase-9 activity. These results suggest that TNF-induced MAP kinase and PI3-kinase/Akt signaling play important roles in protecting BAE cells from TNF cytotoxicity.

L3 ANSWER 28 OF 39 MEDLINE on STN

2001498604 Document Number: 21433667. PubMed ID: 11550089. Extended polyglutamine selectively interacts with caspase-8 and -10 in nuclear aggregates. U M; Miyashita T; Ohtsuka Y; Okamura-Oho Y; Shikama Y; Yamada M. (Department of Genetics, National Children's Medical Research Center, 3-35-31, Taishido, Setagaya, Tokyo 154-8509, Japan.) CELL DEATH AND DIFFERENTIATION, (2001 Apr) 8 (4) 377-86. Journal code: 9437445. ISSN: 1350-9047. Pub. country: England: United Kingdom. Language: English.

AB A growing number of inherited neurodegenerative disorders, including Huntington's disease, have been shown to be caused by the expansion of CAG/polyglutamine repeats. The molecular mechanism underlying these disorders, however, has yet to be clarified. We and others previously demonstrated that caspase-8 was activated by proteolysis in association with the expression of extended polyglutamine. Here, we further analyzed the selectivity of caspases in the process mediated by extended polyglutamine. Among upstream caspases, caspase-10, a close homolog of caspase-8, was also proteolytically activated, but caspase-9 was not. Caspase-8 and -10 were recruited into nuclear aggregates of extended polyglutamine, where at least a fraction of these caspases was converted

to the activated forms. Caspase-8 and -10 were co-immunoprecipitated with polyglutamine only when the polyglutamine was pathologically extended, whereas caspase-2, -3, -6, -7 and -9 were not co-immunoprecipitated with polyglutamine regardless of its size. A dominant-negative form of caspase-8 with a mutation at the catalytic cysteine residue inhibited polyglutamine-mediated nuclear apoptotic phenotype. These results suggest that caspase-8 and -10 are autoactivated as a result of close proximity of the proforms of these molecules that occurs due to aggregate formation, which reveals a novel toxic gain-of-function mechanism for the pathogenesis of CAG-repeat disorders.

L3 ANSWER 29 OF 39 MEDLINE on STN

2001498600 Document Number: 21433663. PubMed ID: 11550085. Caspase-9 processing by caspase-3 via a feedback amplification loop in vivo. Fujita E; Egashira J; Urase K; Kuida K; Momoi T. (Division of Development and Differentiation, National Institute of Neuroscience, NCNP, Kodaira, Tokyo 187-8502, Japan.) CELL DEATH AND DIFFERENTIATION, (2001 Apr) 8 (4) 335-44. Journal code: 9437445. ISSN: 1350-9047. Pub. country: England: United Kingdom. Language: English.

AB In contrast to the autoprocessing of caspase-9, little is known about the biological significance of caspase-9 processing by caspase-3 via a feedback loop in vivo. We prepared antisera against mouse caspase-9 cleavage sites so that only the activated form of mouse caspase-9 was recognized. Using these antisera and caspase-9- and caspase-3-deficient mouse embryonic fibroblasts, we demonstrated that mouse caspase-9 is initially autoprocessed at D(353) and D(368) at low levels during staurosporine-induced apoptosis, whereupon the D(368) and D(168) sites are preferentially processed over D(353) by activated caspase-3 as part of a feedback amplification loop. Ac-DEVD-MCA (caspase-3-like) and Ac-LEHD-MCA (caspase-9-like) cleavage activities clearly showed that caspase-9 autoprocessing was necessary for the activation of caspase-3, whereas full activation of caspase-3 and caspase-9 was achieved only through the feedback amplification loop. This feedback amplification loop also played a predominant role during programmed cell death of dorsal root ganglia neurons at mouse embryonic day 11.5.

L3 ANSWER 30 OF 39 MEDLINE on STN

2001323158 Document Number: 21137954. PubMed ID: 11238216. Butyric acid-induced T-cell apoptosis is mediated by caspase-8 and -9 activation in a Fas-independent manner. Kurita-Ochiai T; Ochiai K; Fukushima K. (Department of Microbiology, Nihon University School of Dentistry at Matsudo, Matsudo, Chiba 271-8587, Japan.. tkurita@mascat.nihon-u.ac.jp) . CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, (2001 Mar) 8 (2) 325-32. Journal code: 9421292. ISSN: 1071-412X. Pub. country: United States. Language: English.

AB Our previous study demonstrated that butyric acid, an extracellular metabolite of periodontopathic bacteria, induced apoptosis in murine thymocytes, splenic T cells, and human Jurkat cells. In this study, we examined whether CD95 ligand-receptor interaction is involved in butyric acid-induced T-cell apoptosis. Flow cytometry analysis indicated that expression of Fas in Jurkat and T cells from peripheral blood mononuclear cells was not affected by butyric acid treatment. Furthermore, the expression of Fas and FasL protein in Western blotting was not affected by butyric acid treatment. Coincubation with blocking anti-Fas **antibodies** prevented Fas-induced apoptosis but not butyric acid-induced apoptosis. Anti-FasL **antibodies** also did not prevent butyric acid-induced apoptosis at any dose examined. Although cytotoxic anti-Fas **antibody** affected butyric acid-induced apoptosis, a synergistic effect was not seen. Time-dependent activation of caspase-8 and -9 was recognized in butyric acid- as well as Fas-mediated apoptosis. IETD-CHO and LEHD-CHO, specific inhibitors of caspase-8 and -9, respectively, completely blocked Fas-mediated apoptosis and partially prevented butyric acid-induced apoptosis. These results suggest that the Fas-FasL interaction is not involved in butyric acid-induced apoptosis and that caspase-8 and -9-dependent apoptosis plays

an important role in butyric acid-induced apoptosis, as well as Fas-induced apoptosis.

L3 ANSWER 31 OF 39 MEDLINE on STN

2000320204 Document Number: 20320204. PubMed ID: 10864208. Induction of apoptosis and activation of the caspase cascade by anti-EGF receptor monoclonal **antibodies** in DiFi human colon cancer cells do not involve the c-jun N-terminal kinase activity. Liu B; Fang M; Schmidt M; Lu Y; Mendelsohn J; Fan Z. (Department of Experimental Therapeutics, The University of Texas MD Anderson Cancer Center, Houston 77030, USA.) BRITISH JOURNAL OF CANCER, (2000 Jun) 82 (12) 1991-9. Journal code: 0370635. ISSN: 0007-0920. Pub. country: SCOTLAND: United Kingdom. Language: English.

AB We previously reported that exposure of DiFi human colon cancer cells to the anti-epidermal growth factor (EGF) receptor monoclonal **antibody** (mAb) 225 resulted in apoptosis, but the mechanisms remain to be elucidated. In the present study, we investigated the effects of a panel of four anti-EGF receptor mAbs, each of which binds to different epitopes of the EGF receptor in DiFi cells, on the induction of apoptosis. We found that each of these mAbs induced apoptosis in DiFi cells. Exposure of DiFi cells to mAb 225 activated the initiation caspase-8, which was detectable between 8 and 16 h after exposure of the cells to the **antibody**. There was also an activation of the initiation caspase-9, which lagged a few hours behind the activation of caspase-8. Exposure of DiFi cells to mAb 225 also activated the execution caspase-3, which was accompanied temporally by evidence of cleavage of a well-characterized caspase-3 substrate, poly(ADP)ribosepolymerase (PARP). Pre-exposure of the cells to the caspase-3-specific inhibitor DEVD-CHO partially reduced the mAb 225-induced PARP cleavage and apoptosis, whereas pre-exposure of the cells to the caspase pan-inhibitor z-VAD-fmk completely inhibited mAb 225-induced apoptosis. Caspases-3, -8 and -9 were not activated in the cell lines in which mAb 225 only induced G1 phase arrest of the cell cycle. In contrast to the apoptosis of DiFi cells induced by ultraviolet irradiation, which strongly activated the c-jun N-terminal kinase-1 (JNK1) and the caspase cascade, mAb 225-induced apoptosis and activation of the caspase cascade in DiFi cells were not associated with activation of JNK1.

L3 ANSWER 32 OF 39 MEDLINE on STN

2000119360 Document Number: 20119360. PubMed ID: 10652256. 4-hydroxynonenal induces a cellular redox status-related activation of the caspase cascade for apoptotic cell death. Liu W; Kato M; Akhand A A; Hayakawa A; Suzuki H; Miyata T; Kurokawa K; Hotta Y; Ishikawa N; Nakashima I. (Department of Immunology, Nagoya University School of Medicine, Showa-ku, Nagoya 466-8550, Japan.) JOURNAL OF CELL SCIENCE, (2000 Feb) 113 (Pt 4) 635-41. Journal code: 0052457. ISSN: 0021-9533. Pub. country: ENGLAND: United Kingdom. Language: English.

AB 4-Hydroxynonenal (HNE), a diffusible product of lipid peroxidation, has been suggested to be a key mediator of oxidative stress-induced cell death. In this study, we partially characterized the mechanism of HNE-mediated cytotoxicity. Incubation of human T lymphoma Jurkat cells with 20-50 microM HNE led to cell death accompanied by DNA fragmentation. Western blot analysis showed that HNE-treatment induced time- and dose-dependent activation of caspase-8, caspase-9 and caspase-3. HNE-induced caspase-3 processing was confirmed by a flow cytometric demonstration of increased catalytic activity on the substrate peptide. HNE treatment also led to remarkable cleavage of poly(ADP-ribose) polymerase (PARP), which was prevented by pretreatment of cells with DEVD-FMK as a caspase-3 inhibitor. The HNE-mediated activation of caspases, cleavage of PARP and DNA fragmentation were blocked by antioxidants cysteine, N-acety-L-cysteine and dithiothreitol, but not by two other HNE-reactive amino acids lysine and histidine, or by cystine, the oxidized form of cysteine. HNE rapidly decreased levels of intracellular reduced glutathione (GSH) and its oxidized form GSSG, and these were also attenuated by the reductants. Coincubation of Jurkat

cells with a blocking anti-Fas **antibody** prevented Fas-induced but not HNE-induced activation of caspase-3. HNE also activated caspase-3 in K562 cells that do not express functional Fas. Our results thereby demonstrate that HNE triggers oxidative stress-linked apoptotic cell death through activation of the caspase cascade. The results also suggest a possible mechanism involving a direct scavenge of intracellular GSH by HNE.

L3 ANSWER 33 OF 39 MEDLINE on STN

2000238091 Document Number: 20238091. PubMed ID: 10773825. Bcl-xL does not inhibit the function of Apaf-1. Newmeyer D D; Bossy-Wetzel E; Kluck R M; Wolf B B; Beere H M; Green D R. (La Jolla Institute for Allergy and Immunology, 10355 Science Center Road, San Diego, CA 92121, USA.) CELL DEATH AND DIFFERENTIATION, (2000 Apr) 7 (4) 402-7. Journal code: 9437445. ISSN: 1350-9047. Pub. country: ENGLAND: United Kingdom. Language: English.

AB Bcl-2 and its relative, Bcl-xL, inhibit apoptotic cell death primarily by controlling the activation of caspase proteases. Previous reports have suggested at least two distinct mechanisms: Bcl-2 and Bcl-xL may inhibit either the formation of the cytochrome c/Apaf-1/caspase-9 apoptosome complex (by preventing cytochrome c release from mitochondria) or the function of this apoptosome (through a direct interaction of Bcl-2 or Bcl-xL with Apaf-1). To evaluate this latter possibility, we added recombinant Bcl-xL protein to cell-free apoptotic systems derived from Jurkat cells and Xenopus eggs. At low concentrations (50 nM), Bcl-xL was able to block the release of cytochrome c from mitochondria. However, although Bcl-xL did associate with Apaf-1, it was unable to inhibit caspase activation induced by the addition of cytochrome c, even at much higher concentrations (1-5 microM). These observations, together with previous results obtained with Bcl-2, argue that Bcl-xL and Bcl-2 cannot block the apoptosome-mediated activation of caspase-9.

L3 ANSWER 34 OF 39 MEDLINE on STN

2000072264 Document Number: 20072264. PubMed ID: 10606248. bcl-X(S)-induced cell death in 3T3 cells does not require or induce caspase activation. Fridman J S; Benedict M A; Maybaum J. (Department of Pharmacology, University of Michigan Medical School, Ann Arbor 48109-0504, USA.) CANCER RESEARCH, (1999 Dec 1) 59 (23) 5999-6004. Journal code: 2984705R. ISSN: 0008-5472. Pub. country: United States. Language: English.

AB Using a tetracycline-regulated expression system, we have shown that expression of bcl-X(s) is sufficient to induce acute cell death in 3T3 cells, and that the manner in which these cells die is both morphologically and biochemically different from Fas/CD95-induced apoptosis. bcl-X(s) expression causes loss of the inner mitochondrial membrane potential (deltapsim) but does not induce caspase activation. Loss of viability, as determined by mitochondrial function and ethidium bromide exclusion, was not inhibited by the broad-spectrum caspase inhibitor zVAD-fmk or by expression of a dominant negative caspase 9 (9DN). However, zVAD-fmk was efficacious in inhibiting cell death triggered by an activating anti-Fas/CD95 **antibody**. In addition, bcl-X(s) does not possess the 5th and 6th alpha-helices (thought to be the membrane-spanning domains in bcl-2, bcl-X(L), and bax) and, therefore, should not be able to form membrane channels, thus eliminating this possible mechanism of action. The finding that bcl-X(s) kills 3T3 cells without caspase activation, along with the absence of membrane spanning domains in bcl-X(s), may, therefore, represent a novel cell death pathway for the pro-death bcl-2 family members.

L3 ANSWER 35 OF 39 MEDLINE on STN

1999443470 Document Number: 99443470. PubMed ID: 10515447. Improved artificial death switches based on caspases and FADD. Fan L; Freeman K W; Khan T; Pham E; Spencer D M. (Department of Microbiology and Immunology, Baylor College of Medicine, Houston, TX 77030, USA.) HUMAN GENE THERAPY, (1999 Sep 20) 10 (14) 2273-85. Journal code: 9008950. ISSN: 1043-0342. Pub. country: United States. Language: English.

AB A number of "suicide genes" have been developed as safety switches for

gene therapy vectors or as potential inducible cytotoxic agents for hyperproliferative disorders, such as cancer or restenosis. However, most of these approaches have relied on foreign proteins, such as HSV thymidine kinase, that primarily target rapidly dividing cells. In contrast, novel artificial death switches based on chemical inducers of dimerization (CIDs) and endogenous proapoptotic molecules function efficiently in both dividing and nondividing cells. In this approach, lipid-permeable, nontoxic CIDs are used to conditionally cross-link target proteins that are fused to CID-binding domains (CBDs), thus activating signaling cascades leading to apoptosis. In previous reports, CID-regulated Fas and caspases 1, 3, 8, and 9 were described. Since the maximum efficacy of these artificial death switches requires low basal and high specific activity, we have optimized these death switches for three parameters: (1) extent of oligomerization, (2) spacing between CBDs and target proteins, and (3) intracellular localization. We describe improved conditional Fas and caspase 1, 3, 8, and 9 alleles that function at subnanomolar levels of the CID AP1903 to trigger apoptosis. Further, we demonstrate for the first time that oligomerization of the death effector domain of the Fas-associated protein, FADD, is sufficient to trigger apoptosis, suggesting that the primary function of FADD, like that of Apaf-1, is oligomerization of associated caspases. Finally, we demonstrate that nuclear-targeted caspases 1, 3, and 8 can trigger apoptosis efficiently, implying that the cleavage of nuclear targets is sufficient for apoptosis.

L3 ANSWER 36 OF 39 MEDLINE on STN

1999310583 Document Number: 99310583. PubMed ID: 10381357. Activation of caspase-3 apoptotic pathways in skeletal muscle fibers in laminin alpha2-deficient mice. Mukasa T; Momoi T; Momoi M Y. (National Institute of Neuroscience, NCNP, Tokyo, Kodaira, 187-8502, Japan.) BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1999 Jun 24) 260 (1) 139-42. Journal code: 0372516. ISSN: 0006-291X. Pub. country: United States. Language: English.

AB dy/dy mice, which carry an unidentified mutation in the Lama2 gene, show dystrophic pathologies similar to those of human congenital muscular dystrophy. Laminin alpha2 deficiency induces apoptosis with DNA fragmentation. Caspases, which are involved in various types of cell death, are sequentially activated through a processing by other members of caspases. By using a cleavage site-directed **antibody** against caspase-3 that specifically reacts with the active form of caspase-3, we immunochemically demonstrated that caspase-3 is activated in the skeletal muscle fiber of dy/dy mice and that some of the activated caspase-3 muscle fibers are TUNEL-positive. Thus the lack of laminin alpha2 signals activates caspase-3, resulting in the apoptosis of muscle fibers.

L3 ANSWER 37 OF 39 MEDLINE on STN

1999255851 Document Number: 99255851. PubMed ID: 10320634. Functional absence of FADD in PLC/PRF/5 hepatoma cells: possible involvement in the transformation to hepatoma in HBV-infected hepatocytes. Suzuki A; Araki T; Miura M; Tsutomi Y. (Drug Safety Research Laboratory, Daiichi Pharmaceutical Co., Ltd., Tokyo 134, Japan.) PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, (1999 May) 221 (1) 72-9. Journal code: 7505892. ISSN: 0037-9727. Pub. country: United States. Language: English.

AB The death receptor Fas transduces apoptotic death signaling upon stimulation by Fas ligand and plays a key role in viral hepatitis. When hepatitis-B virus (HBV) infects hepatocytes, the Fas ligand/Fas system responds as the triggering machinery of hepatitis. However, some HBV-infected cells may circumvent Fas-mediated apoptosis and transform to hepatoma cells, as do PLC/PRF/5 hepatoma cells. Therefore, in the present study, we used PLC/PRF/5 hepatoma cells to investigate this ability to avoid Fas-mediated apoptosis. When the cells were treated with an agonistic Fas **antibody**, they showed resistance to Fas-mediated apoptosis. In contrast, HepG2 cells of the same hepatoma line succumbed. Caspase 3 and 8, which are essential regulators for Fas-mediated cell

death, were expressed in both hepatoma cell lines, but only HepG2 cells showed activation of the caspases. A comparison study of expression of other death-associated factors between PLC/PRF/5 and HepG2 cells revealed no apparent differences. However, Far-Western blotting analysis using the Fas death domain (FDD) showed a significant difference. Molecular weight comparison and immunoblotting analysis revealed that PLC/PRF/5 cells lack the FDD-associated protein FADD. In addition, FDD-injected HepG2 cells showed a resistance to Fas-mediated apoptosis, and PLC/PRF/5 cells acquired Fas-sensitivity by FADD injection. Here, we propose that a functional absence of FADD is one of the pathways for the carcinogenesis of HBV-infected hepatocytes.

L3 ANSWER 38 OF 39 MEDLINE on STN

1998157986 Document Number: 98157986. PubMed ID: 9488720. Caspase-9, Bcl-XL, and Apaf-1 form a ternary complex. Pan G; O'Rourke K; Dixit V M. (Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Mar 6) 273 (10) 5841-5. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Genetic analysis of apoptosis in the nematode Caenorhabditis elegans has revealed the cell death machine to be composed of three core interacting components. CED-4 (equivalent to mammalian Apaf-1) is a nucleotide binding molecule that complexes with the zymogen form of the death protease CED-3, leading to its autoactivation and cell death. CED-9 blocks death by complexing with CED-4 and attenuating its ability to promote CED-3 activation. An equivalent ternary complex was found to be present in mammalian cells involving Apaf-1, the mammalian death protease caspase-9, and Bcl-XL, an anti-apoptotic member of the Bcl-2 family. Consistent with a central role for caspase-9, a dominant negative form effectively inhibited cell death initiated by a wide variety of inducers.

L3 ANSWER 39 OF 39 CAPLUS COPYRIGHT 2003 ACS on STN

1997:761953 Document No. 128:31833 Cloning of human interleukin-1.beta. converting enzyme-like apoptotic protease-6 and its diagnostic and therapeutic applications. Dixit, Vishva M.; He, Wei-wu; Ruben, Steven M.; Kikly, Kristine K. (Smithkline Beecham Corp., USA; Human Genome Sciences, Inc.; University of Michigan). Eur. Pat. Appl. EP 808904 A2 19971126, 44 pp. DESIGNATED STATES: R: BE, CH, DE, DK, FR, GB, IT, LI, NL. (English). CODEN: EPXXDW. APPLICATION: EP 1997-303397 19970519. PRIORITY: US 1996-17949 19960520; US 1996-20344 19960523; US 1996-18961 19960605.

AB Members of the ICE/Ced-3 gene family are likely effector components of the cell death machinery. A novel member of this family designated ICE-LAP-6 is provided. By phylogenetic anal., **ICE-LAP6** is classified into the Ced-3 subfamily which includes Ced-3, Yama/CPP32/apopain, Mch2, and ICE-LAP3/Mch3/CMH-1. Interestingly, **ICE-LAP6** contains an active site QACGG pentapeptide, rather than the QACRG pentapeptide shared by other family members. Overexpression of **ICE-LAP6** induces apoptosis in MCF7 breast carcinoma cells. More importantly, **ICE-LAP6** is proteolytically processed into an active cysteine protease by granzyme B, an important component of cytotoxic T cell-mediated apoptosis. Once activated, **ICE-LAP6** is able to cleave the death substrate poly(ADP-ribose) polymerase into signature apoptotic fragments. Also disclosed are methods for utilizing such ICE LAP-6 for the treatment of a susceptibility to viral infection, tumorigenesis, and to diseases and defects in the control embryogenesis and tissue homeostasis, and the nucleic acid sequences described may be employed in an assay for ascertaining such susceptibility. Agonists and antagonists of ICE LAP-6 may also be used to treat various disease states.

=> s (dixit v?/au or he w?/au or kikly k?/au or ruben s?/au)

L4 7541 (DIXIT V?/AU OR HE W?/AU OR KIKLY K?/AU OR RUBEN S?/AU)

=> s l4 and "ICE-LAP6"
L5 11 L4 AND "ICE-LAP6"

=> dup remove l5
PROCESSING COMPLETED FOR L5
L6 7 DUP REMOVE L5 (4 DUPLICATES REMOVED)

=> d l6 1-7 cbib abs

L6 ANSWER 1 OF 7 MEDLINE on STN
1999214590 Document Number: 99214590. PubMed ID: 10187815. mE10, a novel caspase recruitment domain-containing proapoptotic molecule. Yan M; Lee J; Schilbach S; Goddard A; **Dixit V**. (Department of Molecular Oncology, Genentech, Inc., South San Francisco, California 94080, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Apr 9) 274 (15) 10287-92. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB Apoptotic signaling is mediated by homophilic interactions between conserved domains present in components of the death pathway. The death domain, death effector domain, and caspase recruitment domain (CARD) are examples of such interaction motifs. We have identified a novel mammalian CARD-containing adaptor molecule termed mE10 (mammalian E10). The N-terminal CARD of mE10 exhibits significant homology (47% identity and 64% similarity) to the CARD of a gene from Equine Herpesvirus type 2. The C-terminal region is unique. Overexpression of mE10 in MCF-7 human breast carcinoma cells induces apoptosis. Mutational analysis indicates that CARD-mediated mE10 oligomerization is essential for killing activity. The C terminus of mE10 bound to the zymogen form of caspase-9 and promoted its processing to the active dimeric species. Taken together, these data suggest a model where autoproteolytic activation of pro-caspase-9 is mediated by mE10-induced oligomerization.

L6 ANSWER 2 OF 7 MEDLINE on STN
1999185047 Document Number: 99185047. PubMed ID: 10085063. Caspase-9 can be activated without proteolytic processing. Stennicke H R; Deveraux Q L; Humke E W; Reed J C; **Dixit V M**; Salvesen G S. (The Program for Apoptosis and Cell Death Research, The Burnham Institute, La Jolla, California 92037, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Mar 26) 274 (13) 8359-62. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.
AB The recombinant form of the proapoptotic caspase-9 purified following expression in Escherichia coli is processed at Asp315, but largely inactive; however, when added to cytosolic extracts of human 293 cells it is activated 2000-fold in the presence of cytochrome c and dATP. Thus, the characteristic activities of caspase-9 are context-dependent, and its activation may not recapitulate conventional caspase activation mechanisms. To explore this hypothesis we produced recombinant forms of procaspase-9 containing mutations that disabled one or both of the interdomain processing sites of the zymogen. These mutants were able to activate downstream caspases, but only in the presence of cytosolic factors. The mutant with both processing sites abolished had 10% of the activity of wild-type, and was able to support apoptosis, with equal vigor to wild-type, when transiently expressed in 293 cells. Thus caspase-9 has an unusually active zymogen that does not require proteolytic processing, but instead is dependent on cytosolic factors for expression of its activity.

L6 ANSWER 3 OF 7 MEDLINE on STN
1999094902 Document Number: 99094902. PubMed ID: 9878060. Boo, a novel negative regulator of cell death, interacts with Apaf-1. Song Q; Kuang Y; **Dixit V M**; Vincenz C. (Department of Pathology, The University of Michigan Medical School, Ann Arbor, MI 48109, USA.) EMBO JOURNAL, (1999 Jan 4) 18 (1) 167-78. Journal code: 8208664. ISSN: 0261-4189. Pub. country: ENGLAND: United Kingdom. Language: English.
AB In this report, we describe the cloning and characterization of Boo, a

novel anti-apoptotic member of the Bcl-2 family. The expression of Boo was highly restricted to the ovary and epididymis implicating it in the control of ovarian atresia and sperm maturation. Boo contains the conserved BH1 and BH2 domains, but lacks the BH3 motif. Like Bcl-2, Boo possesses a hydrophobic C-terminus and localizes to intracellular membranes. Boo also has an N-terminal region with strong homology to the BH4 domain found to be important for the function of some anti-apoptotic Bcl-2 homologues. Chromosomal localization analysis assigned Boo to murine chromosome 9 at band d9. Boo inhibits apoptosis, homodimerizes or heterodimerizes with some death-promoting and -suppressing Bcl-2 family members. More importantly, Boo interacts with Apaf-1 and forms a multimeric protein complex with Apaf-1 and caspase-9. Bak and Bik, two pro-apoptotic homologues disrupt the association of Boo and Apaf-1. Furthermore, Boo binds to three distinct regions of Apaf-1. These results demonstrate the evolutionarily conserved nature of the mechanisms of apoptosis. Like Ced-9, the mammalian homologues Boo and Bcl-xL interact with the human counterpart of Ced-4, Apaf-1, and thereby regulate apoptosis.

L6 ANSWER 4 OF 7 MEDLINE on STN

1998157986 Document Number: 98157986. PubMed ID: 9488720. Caspase-9, Bcl-XL, and Apaf-1 form a ternary complex. Pan G; O'Rourke K; **Dixit V M.** (Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Mar 6) 273 (10) 5841-5. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Genetic analysis of apoptosis in the nematode Caenorhabditis elegans has revealed the cell death machine to be composed of three core interacting components. CED-4 (equivalent to mammalian Apaf-1) is a nucleotide binding molecule that complexes with the zymogen form of the death protease CED-3, leading to its autoactivation and cell death. CED-9 blocks death by complexing with CED-4 and attenuating its ability to promote CED-3 activation. An equivalent ternary complex was found to be present in mammalian cells involving Apaf-1, the mammalian death protease caspase-9, and Bcl-XL, an anti-apoptotic member of the Bcl-2 family. Consistent with a central role for caspase-9, a dominant negative form effectively inhibited cell death initiated by a wide variety of inducers.

L6 ANSWER 5 OF 7 MEDLINE on STN

1998258944 Document Number: 98258944. PubMed ID: 9598997. Activation of caspases triggered by cytochrome c in vitro. Pan G; Humke E W; **Dixit V M.** (Department of Pathology, University of Michigan Medical School, Ann Arbor 48109, USA.) FEBS LETTERS, (1998 Apr 10) 426 (1) 151-4. Journal code: 0155157. ISSN: 0014-5793. Pub. country: Netherlands. Language: English.

AB Previous studies have shown that Apaf-1 and caspase-9 in the presence of cytochrome c and dATP can form an initiating complex for an apoptotic protease cascade. We have developed a cytochrome c-dependent in vitro system in which caspases downstream of this initiation complex are activated. The activation of caspase-9 from zymogen form to active dimeric protease requires intrinsic enzymatic activity. In contrast, caspase-3 and caspase-7 zymogens are proteolytically processed by active caspase-9. Activation of the above caspases is blocked by a dominant negative form of caspase-9. The in vitro system displays surprising specificity in that other caspases, including 1, 2, 4, 8, 10, and 13, are not activated.

L6 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2003 ACS on STN

1997:761953 Document No. 128:31833 Cloning of human interleukin-1.beta. converting enzyme-like apoptotic protease-6 and its diagnostic and therapeutic applications. **Dixit, Vishva M.; He, Wei-wu ; Ruben, Steven M.; Kikly, Kristine K.** (Smithkline Beecham Corp., USA; Human Genome Sciences, Inc.; University of Michigan). Eur. Pat. Appl. EP 808904 A2 19971126, 44 pp. DESIGNATED STATES: R: BE, CH, DE, DK, FR, GB, IT, LI, NL. (English). CODEN: EPXXDW. APPLICATION:

EP 1997-303397 19970519. PRIORITY: US 1996-17949 19960520; US 1996-20344 19960523; US 1996-18961 19960605.

AB Members of the ICE/Ced-3 gene family are likely effector components of the cell death machinery. A novel member of this family designated ICE-LAP-6 is provided. By phylogenetic anal., **ICE-LAP6** is classified into the Ced-3 subfamily which includes Ced-3, Yama/CPP32/apopain, Mch2, and ICE-LAP3/Mch3/CMH-1. Interestingly, **ICE-LAP6** contains an active site QACGG pentapeptide, rather than the QACRG pentapeptide shared by other family members. Overexpression of **ICE-LAP6** induces apoptosis in MCF7 breast carcinoma cells. More importantly, **ICE-LAP6** is proteolytically processed into an active cysteine protease by granzyme B, an important component of cytotoxic T cell-mediated apoptosis. Once activated, **ICE-LAP6** is able to cleave the death substrate poly(ADP-ribose) polymerase into signature apoptotic fragments. Also disclosed are methods for utilizing such ICE LAP-6 for the treatment of a susceptibility to viral infection, tumorigenesis, and to diseases and defects in the control embryogenesis and tissue homeostasis, and the nucleic acid sequences described may be employed in an assay for ascertaining such susceptibility. Agonists and antagonists of ICE LAP-6 may also be used to treat various disease states.

L6 ANSWER 7 OF 7 MEDLINE on STN DUPLICATE 1

96279246 Document Number: 96279246. PubMed ID: 8663294. **ICE-LAP6**, a novel member of the ICE/Ced-3 gene family, is activated by the cytotoxic T cell protease granzyme B. Duan H; Orth K; Chinnaiyan A M; Poirier G G; Froelich C J; **He W W**; **Dixit V M**. (Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA.) JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Jul 12) 271 (28) 16720-4. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Members of the ICE/Ced-3 gene family are likely effector components of the cell death machinery. Here, we characterize a novel member of this family designated **ICE-LAP6**. By phylogenetic analysis, **ICE-LAP6** is classified into the Ced-3 subfamily which includes Ced-3, Yama/CPP32/apopain, Mch2, and ICE-LAP3/Mch3/CMH-1. Interestingly, **ICE-LAP6** contains an active site QACGG pentapeptide, rather than the QACRG pentapeptide shared by other family members. Overexpression of **ICE-LAP6** induces apoptosis in MCF7 breast carcinoma cells. More importantly, **ICE-LAP6** is proteolytically processed into an active cysteine protease by granzyme B, an important component of cytotoxic T cell-mediated apoptosis. Once activated, **ICE-LAP6** is able to cleave the death substrate poly(ADP-ribose) polymerase into signature apoptotic fragments.

```
=> s anti-lap6
L7            0 ANTI-LAP6

=> s anti-caspase 9
L8            4 ANTI-CASPASE 9

=> dup remove l8
PROCESSING COMPLETED FOR L8
L9            1 DUP REMOVE L8 (3 DUPLICATES REMOVED)

=> d l9 cbib abs
```

L9 ANSWER 1 OF 1 MEDLINE on STN DUPLICATE 1

2001429666 Document Number: 21369983. PubMed ID: 11395480. Bone morphogenetic protein-2 promotes osteoblast apoptosis through a Smad-independent, protein kinase C-dependent signaling pathway. Hay E; Lemonnier J; Fromigue O; Marie P J. (Laboratory of Osteoblast Biology and Pathology, INSERM U 349, Affiliated CNRS, Lariboisiere Hospital, 75475

Cedex 10 Paris, France.) JOURNAL OF BIOLOGICAL CHEMISTRY, (2001 Aug 3) 276 (31) 29028-36. Journal code: 2985121R. ISSN: 0021-9258. Pub. country: United States. Language: English.

AB Bone morphogenetic protein-2 (BMP-2), a member of the transforming growth factor-beta (TGF-beta) family, regulates osteoblast differentiation and bone formation. Here we show a novel function of BMP-2 in human osteoblasts and identify a signaling pathway involved in this function. BMP-2 promotes apoptosis in primary human calvaria osteoblasts and in immortalized human neonatal calvaria osteoblasts, as shown by terminal deoxynucleotidyl transferase-mediated nick end labeling analysis. In contrast, TGF-beta 2 inhibits apoptosis in human osteoblasts. Studies of the mechanisms of action showed that BMP-2 increases the Bax/Bcl-2 ratio, whereas TG beta-2 has a negative effect. Moreover, BMP-2 increases the release of mitochondrial cytochrome c to the cytosol. Consistent with these results, BMP-2 increases caspase-9 and caspase-3, -6, and -7 activity, and an **anti-caspase-9** agent suppresses BMP-2-induced apoptosis. Overexpression of dominant-negative Smad1 effectively blocks BMP-2-induced expression of the osteoblast transcription factor Runx2 but not the activation of caspases or apoptosis induced by BMP-2, indicating that the Smad1 signaling pathway is not involved in the BMP-2-induced apoptosis. The proapoptotic effect of BMP-2 is PKC-dependent, because BMP-2 increases PKC activity, and the selective PKC inhibitor calphostin C blocks the BMP-2-induced increased Bax/Bcl-2, caspase activity, and apoptosis. In contrast, the cAMP-dependent protein kinase A inhibitor H89, the p38 MAPK inhibitor SB203580, and the MEK inhibitor PD-98059 have no effect. The results show that BMP-2 uses a Smad-independent, PKC-dependent pathway to promote apoptosis via a Bax/Bcl-2 and cytochrome c-caspase-9-caspase-3, -6, -7 cascade in human osteoblasts.

=>

---Logging off of STN---

=>
Executing the logoff script...

=> LOG Y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	47.41	47.62

DISCOUNT AMOUNTS (FOR QUALIFYING ACCOUNTS)	SINCE FILE ENTRY	TOTAL SESSION
CA SUBSCRIBER PRICE	-2.60	-2.60

STN INTERNATIONAL LOGOFF AT 15:38:21 ON 10 NOV 2003